THE MICROBIAL COMMUNITIES AND NUTRIENT AVAILABILITY IN PRE AND POST HARVESTED LODGEPOLE PINE STANDS OF WEST-CENTRAL ALBERTA

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By

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ABSTRACT

All organisms within a forested system play a role in the biogeochemical cycle, not only within the forest but also within the global community. Soil microorganisms are a vital part of this cycle, as they sequester or make nutrients available for the development of the forest environment. When a disturbance event occurs, changes to the environment occur; however, it is unclear how these changes affect the soil's microbial community. This 2-year (2007 and 2008) study was carried out to obtain a preliminary assessment of the microbial community structure and nutrient (nitrogen and phosphorus) availability within lodgepole pine stands of the Boreal Plain ecozone in west-central Alberta. Six stands of different ages were selected to determine the differences between pre and post harvest. Nutrient flux measurements were conducted using plant root simulator (PRS) probes to investigate the changes in nutrient availability. The microbial community structures were determined using two biochemical methods. The first one was a community level physiological profile (CLPP), which provides information concerning the functional characteristics of the microbial communities. Phospholipid fatty acid (PLFA) analysis provides information about the physiological characteristics of the microbial community.

Analysis of the PRS probes results varied for the two nutrients: phosphorus (P) and nitrogen (N). Nitrogen availability was determined by examining the fluxes of ammonium and nitrate to the PRS probes. These did not show a strong relationship between the different aged stands during 2007 or 2008. In addition, no statistical difference was shown between the 2007 and 2008 data compared to the LFH or the mineral soil of the stands. Phosphorus, however, did show a potential trend where there was an initial increase of available P after harvest and then a gradual decrease, as the forest stands matured. This was strongly observed within the LFH, while there was a slight increase in the mineral layer. These trends remained consistent over the

two-year period showing a gradual decrease in P flux to the PRS probes as a stand aged even in just one year.

The microbial communities did not show a strong change after a forest-harvesting event. When examining the functional groups, there was a drastic shift in the LFH layer microbial community over the first sampling season. This change remained the same within the beginning of the second sampling year. This shift occurred in all stands due to an environmental factor, which was suspected to be the increase in moisture during the season. The change in the microbial communities was not observed, however, in the mineral layer of the soil when the functional structure was examined. When the physiological composition of the microbial communities was observed, though, using PLFA, it was apparent that the physiological characteristics of the microbial community had changed in the mineral soil. Furthermore, no physiological change was observed in the microbial communities of the LFH, only a functional change.

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I could not have accomplished this without you all.

Dedication

I dedicate this thesis to a man who worked all his life to provide the best for his family and who always believed his children would live diverse lives.

CASIAN MASCARENHAS

1944 – 2002

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
AWCD	Average well colour density
CLPP	Community level physiological profile
FMA	Forest Management Area
FORWARD	Forest Watershed and Riparian Disturbance
LF	Lower foothills
MRPP	Multiresponse permutation procedure
NMS	Non-parametric multidimensional scaling
OM	Organic matter
PCA	Principal component analysis
PLFA	Phospholipid fatty acid
PRS	Plant root simulator

CHAPTER 1
Introduction

Canada contains 10% of the world's forests (National Resource Canada, 2009). The boreal forests alone make up 77% of the forested land in Canada (Smith et al., 2003b) and more than 90% of these forests are used for commercial products like lumber or paper (National Resource Canada, 2009). As a result, these forest ecosystems are managed in such a way as to prevent and mitigate damage, which would reduce destruction by pests, disease and from anthropogenic sources. The management of these sites encourages a rapid recovery after harvest with a minimal loss of nutrients and provides uncompromised productivity for future trees.

Recently, there has been an increased focus on sustainable ecological management within forestry (Barnes et al., 1998). The scientific community wishes to better understand the implications of clear-cutting on different soil characteristics (Adams et al., 1991; Bossio and Scow, 1998). The Forest Watershed and Riparian Disturbance (FORWARD) project was established to develop innovative tools for forest industries that want to predict and manage their practices (Smith et al., 2003a). One aspect of FORWARD is to understand the relationship between forest management practices and soil biogeochemical cycles. The biological, chemical and physical characteristics of soil are complex, making it difficult to form a reliable soil quality index for managing forest soils (Staddon et al., 1998). It is well documented, however, that the clear cutting of trees and the removal of above ground biomass alter the underlying soil characteristics. Whitson et al. (2005a)

demonstrate this very clearly and show increased soil moisture and temperature after clear-cutting.

One of the major biological components of soil affected by clear-cutting is the microbial community. Microbial communities within soils are very important as they drive the biogeochemical cycles on local and global scales (Zak et al., 2006a). They play an important role in mobilizing and sequestering nutrients within the soil either by increasing or limiting nutrient availability for plants and other organisms. The heterotrophic microbial community in soil is important for the control of ecosystem carbon and nitrogen cycling (Zak et al., 2006b). The microbial community may potentially be the link between the function of soil ecosystems and their plant community structure (Zak et al., 2003).

It is well documented that changes to the soil microbial communities occur after disturbance events in a forest system. Smith et al. (2008) found that, after an extreme fire event, the top layer of soil was removed, causing a dramatic 74% loss in the microbial populations. Temperatures as low as 70 ˚C can kill some bacteria found in soils (Raison, 1979). Recently, JimÈnez EsquilÌn et al. (2007) found that soil microbes within a microsite in the vicinity of a burn pile may be adversely affected. They also found that the microbial community's recovery at the microsite took more then 15 years for it to reach a similar state to the surrounding area (JimÈnez EsquilÌn et al., 2007).

Fire is not the only disturbance factor that can alter soil microbial communities. An increase in soil moisture is also linked to changes in the size and structure of the microbial community. For example, several studies show that increased water levels and saturation of the soil can be linked to an increase in anaerobic microbial populations (Bossio et al., 1998; Chanasyk et al., 2003). It was also noted that both heterotrophic and aerobic

microbial populations within these microbial communities tend to decrease under saturated conditions. Changes in microbial community structure can also significantly affect nutrient availability. Generally, it has been observed that with an increase in moisture, there is an increase in nitrate availability and anaerobic respiration, which can cause a dramatic increase in nitrogen loss into the environment in the form of N_2O and NOx (Schimel, 1998).

Naturally occurring events are very difficult to control, but their anthropogenic counterparts such as a moisture increase due to the removal of above ground vegetation or soil compaction caused by heavy machinery, can be controlled and mitigated to reduce their effects. A good example of these anthropogenic impacts is forest harvesting. It is well documented that harvesting impacts the soil microbial communities. Current practices do mitigate some of the impact such as from soil compaction or the loss of forest floor biomass. Using heavy harvesting machinery during the winter mitigates soil compaction because the soil is frozen at that time (Mariani et al., 2006; Silvia et al., 2006). In addition, the prescribed burning of ground cover allows for a control fire which reduces the potential volatility of the forest stands (Chanasyk et al., 2003; Lertzman et al., 2002). In the case of burn piles, forestry companies wait until the winter to burn these piles of wood in order to prevent large-scale fires and reduce the formation of a microsite (JimÈnez EsquilÌn et al., 2007), a localized area where the microbial community is reduced.

Forest harvesting causes changes in soil characteristics: for example, decreases in pore space and increased soil temperature alters the microbial community structure. Some populations may increase while others may decrease, as well, in some cases, populations may be lost (Pietikäinen and Fritze, 1995). It is not clear how long it takes for these soil

microbial communities to recover to a community structure similar to how it was before a harvesting event (Hassett and Zak, 2005). These changes to the microbial community are linked to physical, biological and chemical changes within the soil after the area has been harvested.

In this research, it is hypothesized that the pre and post harvest forest stands have significantly different levels of available nutrients and soil microbial community structures. The hypothesis was tested through an *in situ* experiment to determine the nutrient flux within pre and post harvest stands. Two laboratory experiments were carried out: one to examine the functional characteristics of the microbial communities within the pre and post harvest stand using community level physical profile (CLPP) and the second, to determine the physiological structure of the microbial community, based on phospholipid fatty acid (PLFA) analysis. These laboratory experiments complemented the in *situ* study.

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CHAPTER 2
Literature Review

2.1 Forests

The world trend toward urbanization and industrialization is causing forested areas to be destroyed to form agricultural land and expand urban centers. Currently, Canadian forests cover 418 million ha of land of which 218 million ha are used for commercial products (Cofer et al., 1996).

Forests play a major role in the earth's biogeochemical cycles. They act as a sink and source for several macronutrients such as nitrogen, phosphorus, carbon and oxygen, which are the basic building blocks of all life (Botkin et al., 2006). Further, forests provide a specialized environment that supports many organisms such as: plants, animals and microorganisms like fungi and bacteria (Lindenmayer and Franklin, 1997). Forested systems have a large amount of above ground biomass and are able to create an environment that is not directly affected by extreme conditions such as evaporation due to intense solar radiation.

Many different plant species are found in a forest and vary between different forests. The dominant forest ecosystems in Canada are the Boreal forests, which house a number of different tree species such as black spruce, lodgepole pine and trembling aspen as well as surface plants such as lichen, mosses, shrubs, herbs and grasses. The moist environment provides the water that plants require, a stable medium for growth and ample nutrients and shade. The large trees provide a canopy cover that causes the ground vegetation to be

specialized for specific wavelengths of sunlight in the middle of the infrared spectral region (Kuusk et al., 2004). Thus, these specialized plants can propagate and grow.

Forest ecosystems also provide specialized environments for microbial development, specifically, the development of different microhabitats (Rich et al., 2003). These microhabitats vary in their moisture content, soil temperature, litter fall and solar radiation intensity. Slight variations in these variables throughout a forest can cause varied microorganism populations to develop (Arnold et al., 1999). A good example of this is the heterotrophic bacteria. When extensive rainfall or flooding occurs within a forested area, oxygen depletion occurs in the soil, which then becomes anoxic so that oxygen dependent microbes die (Waldrop et al., 2000; Peter Mayer and Conrad, 1990). Some heterotrophic microbes such as denitrifying bacteria are able to use an alternative chemical source for respiration. In many cases, these bacteria start to reduce the nitrate for respiration, which enables their survival (Wrage et al., 2001). This ability is highly specialized and not commonly present in all microorganisms.

Soil microbial communities are important to the growth and sustainability of forests. Microbial communities play an important role in the biogeochemical cycle (Wittman et al., 2004). Changes within the forest such as a harvesting event can alter the microbial communities. Harvesting damages the forest's environment by altering the characteristics of the forest floor, which in turn can alter the structure and diversity of the soil microbial communities (Fraterrigo et al., 2006). Changes in the microbial community are linked to nutrient flux in stands and cut blocks. As a result, the current harvesting practices can influence both the nutrient balance and the forests biological equilibrium.

2.1.1 Modern Forestry Management Techniques

Several tree-harvesting techniques have been developed over the past century to reduce the damage to soil and lower vegetation. These techniques have all been designed to reduce soil erosion, nutrient loss, as well as to maintain similar soil characteristics to that of the pre harvest stand (Palviainen et al. 2004; Smith et al. 2003). Clear cutting is one of the major methods of harvesting. However, other methods such as selective cutting or shelterwood harvesting have been considered for their low impact effects (Bradley et al., 2001; Olsson et al., 1996; Smith et al., 2008). When taking into account both the cost and benefits of the different harvesting methods, clear-cutting is the most efficient method. Forestry companies in North America are thus trying to mitigate for the adverse affects of clear-cutting. To reduce soil damage by heavy machinery in the form of erosion and compaction, the forestry industry harvests during the winter season when the soil is frozen. Therefore, surface vegetation is also minimally affected since it is generally dormant or dead; whereas any vegetation that is alive during the winter, generally can withstand significant stress (Botkin et al., 2006). An example of this is the rapid regrowth and reproduction of new tissue of grasses and lower vegetation like raspberries (Kulmala et al., 2009).

2.1.2 Site Preparation

In addition to harvesting techniques, site preparation is another means of mitigating the impact of harvesting. Previous techniques removed whole trees from the stand and then removed the branches from the stand offsite (Olsson et al., 1996). None of the branch material, also known as slash, was returned to the stand. Studies from Europe show that leaving the slash on site is a means of returning some of the nutrients to the soil (Palviainen

et al., 2004). In addition, forestry companies have tried burning the waste plant residue in order to rapidly return nutrients to the soil. The negative effect of this method is that, when burning occurs, a large amount of nutrients is lost from the system in a gaseous form and released into the air rather than returned to the soil (JimÈnez EsquilÌn et al., 2007). The most vulnerable nutrient in this situation is nitrogen, which is readily volatized into the atmosphere at temperatures common to forest fires. When the nutrients are returned to the soil, the vegetation is unable to rapidly use all the nutrients so that large quantities are lost by leaching and run off (Staddon et al., 1998). There are many other methods of site preparation like scalping, mixing, mounding, burning and herbicide treatment. Each of these methods has their own advantages. Scalping provides a vertical profile within the stand creating berms (raised location good for planting on wet sites), hinges (favourable for ground level planting) and furrows (depressed locations good for dry sites) (McMinn and Hedin, 1990). Mixing exposes the mineral layer, which allows for better heat exchange between the ground and air, reduces the frost injury to the seedlings (Cortini et al., 2010; Örlander et al. 1990). Mounding creates a layer of soil above ground were the plants are safe from flood damage (Cortini et al., 2010; Örlander et al. 1990). Burning of the stand reduces the understory vegetation and competition the seedlings would face during re-establishment of the stand (Cortini et al., 2010; Örlander et al. 1990). In addition, herbicide can also be a great means of preparing for planting by reducing specific groups of plant species within the stand, like grasses (Cortini et al., 2010; Örlander et al. 1990).

A current technique of site preparation is a mixture of the two techniques previously described and is the current method being used in West Central Alberta. This technique is the rake and burn method. This method creates a burn pile made up of only the larger limbs

of the tree, while the smaller tree branches and needles in the case of conifers are left on the stand. The idea behind this is that the smaller material will naturally decompose over time and gradually return nutrients to the soil. In this manner, not only will the plants be able to better use the nutrients, but also a rapid loss of nutrients through leaching and runoff will be reduced. The large branches, however, are burned thus making valuable nutrients available for the plants right after harvest. There is some leaching and runoff of nutrients, but not at levels as high as those in the previous method (Bhatti et al., 2000)

2.2 Effect of forest harvesting on nutrient cycling

Nutrients are the building blocks of all organisms. Nitrogen and phosphorus are the two main nutrients that influence the development and sustainability of the microbial community within a forest system. Forest harvesting can influence the mobility and availability of these nutrients. Before forests are harvested, a great deal of competition for the nutrients occurs between soil microorganisms and plants. Plants with extensive root systems tend to out compete soil microbes for nutrients, which causes a circumstance where nutrients become limited for the microbes. The increasing competition between microbes for nutrients causes the development of a dynamic environment (Hodge et al., 2000). After harvesting, with the removal of all large vegetation that took up most of the nutrients, initially, there is an abundance of nutrients available for microorganisms (Piirainen et al., 2004). Due to this over abundance, leaching can occur and cause high levels of nutrient loss from the environment through both ground water and surface runoff (Grip 1982; Rosén et al. 1996; Ahtiainen and Huttunen 1999).

2.2.1 Mobilization and movement of nitrogen

Nitrogen is a macronutrient that is found in two major forms: ammonium (NH_4^+) and nitrate (NO_3^-). These forms of nitrogen are limiting factors in plant and microorganism growth and development (Botkin et al., 2006). When these forms of nitrogen are available, they are rapidly immobilized and taken up by competing plants and microbes. However, some instances exist where nitrogen becomes mobilized within the environment (Olsson et al., 1996). This is generally due to changes in soil characteristics such as water content, organic matter (OM), pH, and temperature (Speir et al., 1999), all of which affect the nitrogen cycle (Figure 2.2.1).

The water content of the soil can influence the movement of nitrogen within the soil. Lowered water content slows the movement of nutrients and increases the adsorption of NH_4^+ onto the clay molecules (Schelsinger et al., 1990). When the soil is wet, some forms of nitrogen are able to dissolve into solution and the denitrification or nitrification reactions can occur, which enable nitrogen forms to become mobile. The saturation of soils can result in increased mobility of nitrogen in the form of NO_3^-. In turn, saturation of the soil creates anoxic conditions and enables anaerobic bacteria, such as denitrifyers, to become the dominant microbial population. These denitrifyers convert NO_3^- into the form of NO_2^-, NO, N_2O and N_2 for a means of respiration (Wallenstein et al., 2006).

Organic matter and water are very different in their effects on soil microbial communities. When there is a large quantity of organic matter, the microorganisms decompose the OM into forms that they can use for their growth. The increase in available nutrients allows the microbial population to increase. As a result, more microbes form and the rate of decomposition increases. This increases the decomposition of OM causing the liberation of NH_4^+ and making it available in the soil. Thus, an increase in the

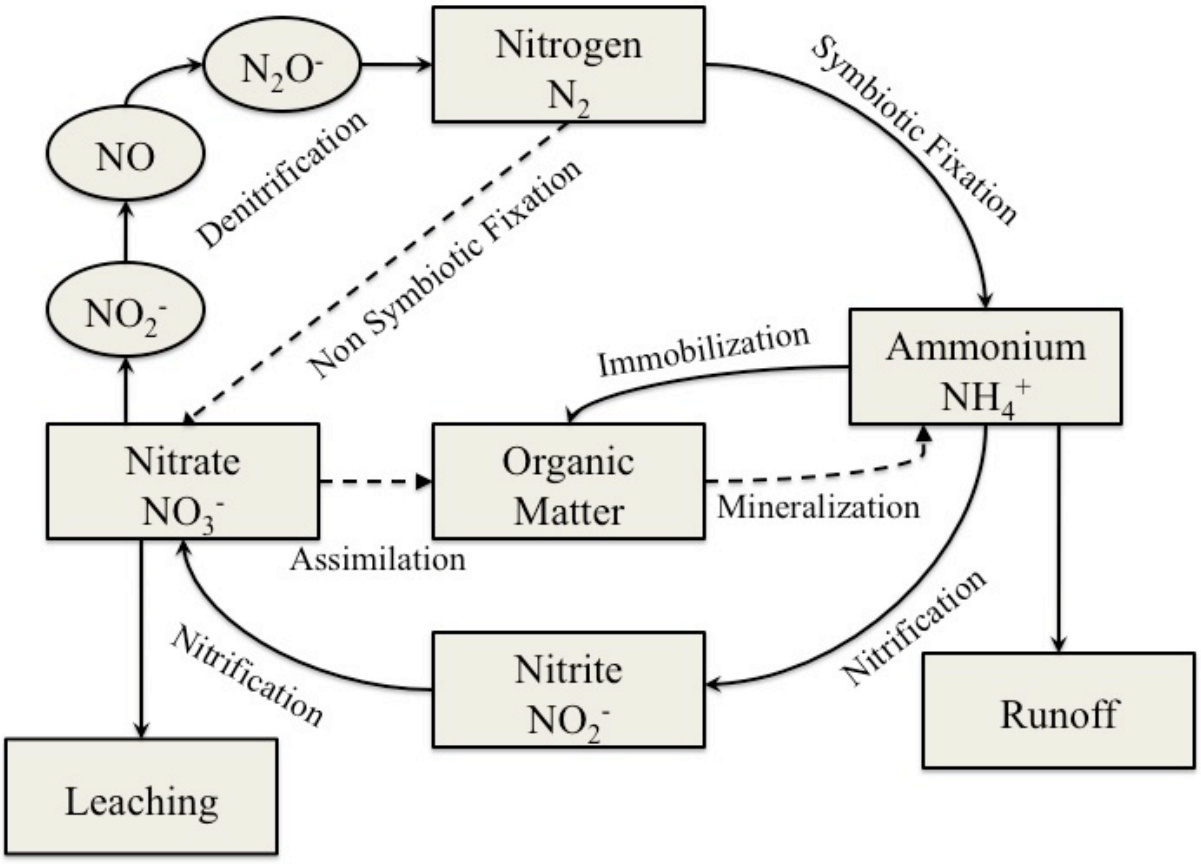

Figure 2.2.1 – The soil nitrogen cycle which indicates the process and the different forms of nitrogen that can be found within the soil at one time. This diagram is a compilation of multiple soil nitrogen cycle diagrams (Paul and Clark, 1989; Barnes et al., 1998; Likens and Bormann, 1999; Wetzel, 2001; Botkin et al., 2006).

mineralization of NH_4^+ can cause an increase in the rate of nitrification and formation of NO_3^- (Schimel and Bennett, 2004). Nitrate is found in acid rain and soils with a low pH. This acidification of the soil causes protons (H^+) to replace base cations in the soil and make these ions mobile. Studies indicate that acid rain can have beneficial effects for forested systems that have a high buffering capacity and limited available nitrogen (Raison, 1979). The acidification liberates NH_4^+ from its bond with the soil particles (Reuss et al., 1987). When liberated this increases the concentration of mobile N (Reuss et al., 1987). A study by Likens et al. (1996) demonstrated that productivity increases within the forest stand with increases in acid rain. Even though, there are some benefits associated with decreasing the pH of a system, a secondary effect in poorly buffered systems is the loss of nutrients. Ammonium is one of the major nutrients that becomes mobile within the soil as the pH decreases. This results in the leaching of NO_3^- and NH_4^+ from the system into an adjacent stream or river system.

High temperature can also be fairly detrimental to nitrogen cycling because many nitrifying bacteria are temperature sensitive and can die at around 54 °C (Raison, 1979). In situations such as fire, nitrogen can be volatilized and directly removed from the system causing the environment to be increasingly nitrogen limited (Chanasyk et al., 2003). At the other extreme of the temperature scale, extreme cold is generally not very beneficial for nitrogen cycling either. Under cold conditions, many microbial processes become very slow, as microbes are unable to function properly. As a catalyst for the breakdown and movement of nutrients within the soil, changes within the microbial process can alter the nutrient movement (Morgan-Kiss et al., 2006). These changes in temperature; however, do not affect the long-term nitrogen cycle.

Changes in water content, OM, pH and temperature all occur after a harvesting event and must be considered when managing a forested ecosystem. Water content tends to increase after harvesting because the trees that were utilizing the water are no longer there and the water is now saturating the soil (Schnürer, 1986). The pH can be adversely affected, especially, in coniferous stands where the needles remain on the floor. Conifer needles are acidic and when they break down, the acidity of the soil can increase (Cofer et al., 1996). Temperature also tends to change drastically once the canopy cover has been removed. These changes in environmental factors can thus alter the nitrogen cycle within the stands.

2.2.2 Phosphorus movement in soils

Phosphorus behaves very differently from nitrogen in its relationship to the soil. Phosphorus is generally found as phosphate (PO_4^{3-}) and is highly immobile within the soil while the $H_2PO_4^-$ form of P is mobile in acidic conditions and HPO_4^{2-} in alkaline conditions (Wetzel, 2001). In a mature forest stand, phosphorous is rapidly immobilized, (Whitson et al., 2005b) but it is not a limiting factor in most terrestrial environments. Changes in environmental variables such as temperature, water content, and pH can influence the phosphorus cycle in soils (Figure 2.2.2).

Fluctuations in temperature can influence the rate at which the phosphorus cycle occurs. At high temperatures, such as during a fire, microorganisms and extracellular enzymes become denatured so that part of the P cycle is slowed however the rapid breakdown of surrounding vegetation will increase labile P (Certini, 2005). At very low.

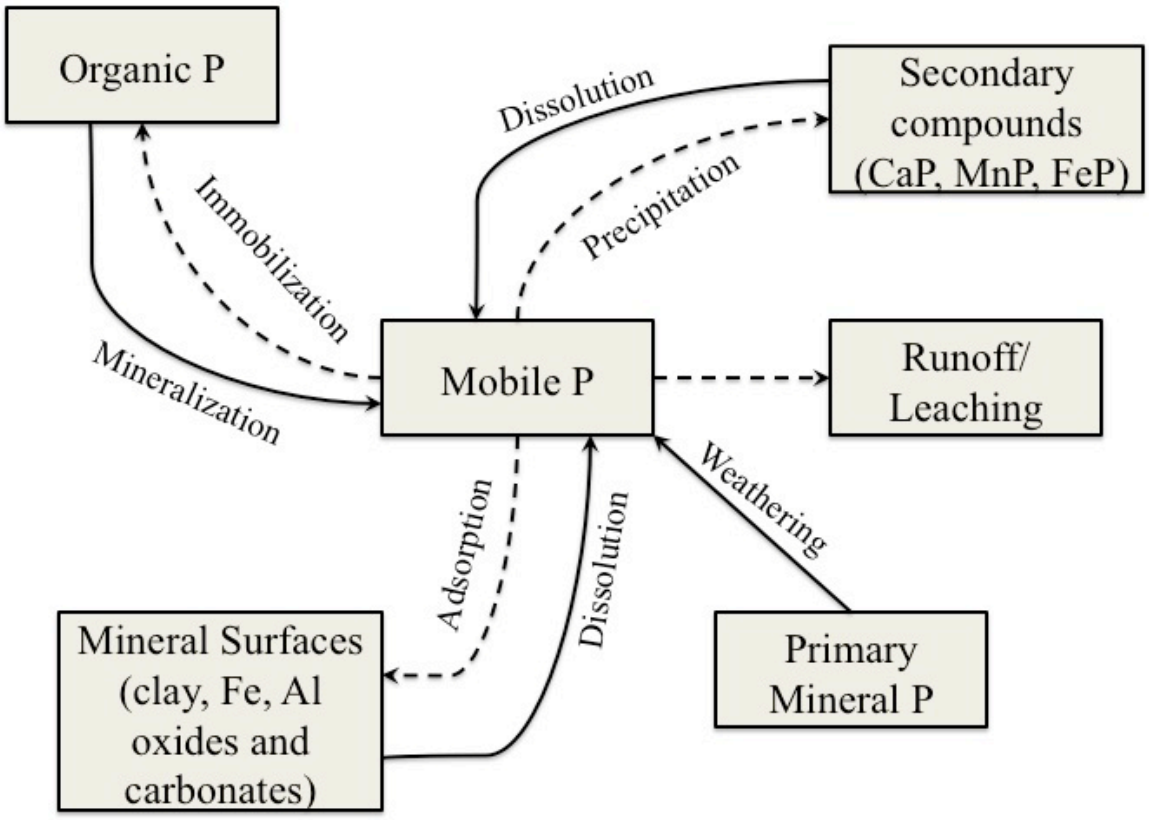

Figure 2.2.2 – Soil phosphorus cycle, showing the directional movement of phosphorus between different structural states. This diagram is a compilation of multiple soil phosphorus cycle diagrams (Paul and Clark, 1989; Barnes et al., 1998; Likens and Bormann, 1999; Wetzel, 2001; Botkin et al., 2006).

temperatures, all catabolic activity decreases and a slowing of the chemical processes in the P cycle occur. Water content, generally, does not alter the phosphorus cycle however, under extreme conditions of water saturation, the phosphorus cycle may be influenced by the lack of oxygen and reduced aerobic microorganisms population (Wetzel, 2001). pH is the major characteristic that influences the availability or mobility of phosphorus within the soil. Phosphorus is highly immobile, and is generally adsorbed to the soil mineral surface or secondary compounds in the soil (Figure 2.2.2). Under low pH (acidic) conditions, immobile P is converted to mobile P (Schachtman et al., 1998). H_3PO_4, a neutral chemical, is formed under the acidic conditions and is able to dissolve within the soil water (Schachtman et al., 1998). Therefore, the acidification of the soil results in an increase in a mobile form of phosphorus available to be taken up by microorganisms and plants. However the increased mobile phosphorus tends to be leached from the environment (Gahoonia et al. 1994).

2.3 The interaction of forest harvesting (clear-cutting) and soil microbial communities

Bacteria and fungi are two major groups of microorganisms that drive part of the biogeochemical cycle within the soil (Brimecombe et al., 1998). It is believed that clear-cutting may impact the diversity of the microbial communities within the soil (Hassett and Zak, 2005; Kieft et al., 1987b). Microbial communities and nutrients are affected by similar soil characteristics (Schimel and Bennett, 2004). Water content, OM, pH and temperature alter the microbial community structure.

2.3.1 Environmental impact on forest soil microbial communities

Water is an important variable in the development of soil microbial communities (Schnürer, 1986), as nutrients are transported though the water to and from the microbes. Under low moisture content, microbial populations decrease because their accessibility to nutrients becomes limited by the inability of the nutrients to move through the soil. This is due to the lack of an aqueous solution, which is vital for nutrients transfer within soil. The lack of water also reduces the microbial population through dehydration. Some groups of microorganisms have overcome this by increasing their intercellular solutes to prevent dehydration (Kieft et al., 1987b).

Saturation can also be detrimental to microbial communities. The soil can become anoxic, which results in increased death for oxygen dependent microorganisms, while allowing anaerobic microbes to survive (Wetzel, 2001). Anoxic conditions reduce the diversity of the microbial communities and its biomass (Friedel et al., 2006). Saturation is a common occurrence after a stand has been harvested (Bormann et al., 1968) because there are no longer trees present to take up the available moisture. The increased water then remains within the soil, increasing and, in some situations, exceeding the soil's water holding capacity.

Organic matter is another soil characteristic that can alter the microbial community. The organic matter provides the ideal environment for the development of bacteria and fungi. The large amounts of fibric material are caused by the decomposition of plant material and mosses. This provides highly absorbent material that retains moisture over a long period, which creates a favorable environment for microbial development. The organic layer within the soil also provides macro and micronutrients from decomposing plant matter, which further enables microbial development. A common decomposer is

Pseudomonas fluorescens (Brimecombe et al., 1998), which breaks down an organic nitrogen molecule and through multiple processes converts it into nitrate (Figure 2.2.1), which is then used in the development of amino acids by plants and soil microorganisms (Schimel and Bennett, 2004).

pH also influences the development and survival of microbial communities within forested areas. Initially, microbial communities increase as nutrients become available within the system. Under unfavorable pH conditions, these communities can suffer. Low soil pHs can also influence enzyme activity, by inhibiting the enzyme (Speir et al., 1999) and other microorganism proteins (Bååth, 1998), most likely by denaturing the proteins.

Local temperatures can influence the soil microbial community and activity. Under low temperatures, soil microbial activity is reduced and some microorganisms are unable to survive in cold environments. However, some microbial communities have adapted to survive at extremely cold temperatures such as those found in the Arctic and the Antarctic environments. These microorganisms have developed two major adaptation mechanisms to overcome these cold environments. For instance, some microorganisms are able to decrease their body's freezing point by evolutionary changes such as the production of antifreeze proteins within the cells and /or secondly, by having a more fluid cell membrane with the use of polyunsaturated short-chains (Morgan-Kiss et al., 2006).

High temperatures also affect the development of microorganisms. Under high temperatures, enzymes tend to be denatured which can reduce the microbial activity of a large portion of the microbial population and in some extreme cases, organism death can occur. At a temperature of 70 °C, a partial sterilization of soil occurs and many microorganisms die (Raison, 1979). Microorganisms that face extreme high temperatures

for long periods have also adapted to the environment by producing “ether lipids derived from diphytanyl-glycerol or its dimmer di(biphytanyl)-diglycerol”, which are highly resistant to high temperatures (Stetter, 1999). After harvesting, these extreme temperatures are generally only found within the soils below your burn piles; however, minor decreases or increases within the forest soils can alter the structure of the microbial community, as better-adapted microbes become dominant.

Competition is a major factor that influences change in microbial community structure. In some cases, specific microbial populations can substantially decrease in size with the loss of nutrients, as they are unable to compete with other organisms for the nutrients. In addition, over time plants over compete for these nutrients by having a long-term root system, while the microbial communities die and regrow each year.

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CHAPTER 3
Nitrogen and Phosphorus Availability in Pre and Post Harvest Stands of West Central Alberta

3.1 Preface

Nutrient retention during and after clear cutting events plays an important role in being able to maintain a sustainable forest and the forest as a global resource. During forest harvesting and the re-establishment of a forest, the available nutrient concentrations change in the soil, which can reduce the health of the forest stand and influence its watershed by increasing the nutrient levels within the streams and rivers. This study examines soil nitrogen and phosphorus availability by utilizing plant root simulator flux measurements over a two-year period for five stands at different times after harvesting and re-growth.

3.2 Introduction

Coniferous tree species are a major contributor to wood production in west-central Alberta, and account for ~60% of what is produced (McCready, 2009). Lodge-pole pine is one of the major conifers used in commercial forestry products such as the production of composite board furniture, windows, doors and shutters, paneling, edge-glued shelving, siding, mouldings, and other architectural millwork (Jozsa and Middleton, 1994). A forestry company's ability to regenerate a stand with a minimal loss of nutrients after timber harvesting is very important for the survival of many forestry companies (Millar Western Forest Products, 2006). Consequently, sustainable harvesting is an important

issue for this industry. To be sustainable, forestry companies need to understand nutrient cycling within pre and post harvest stands. Nitrogen and phosphorus are the two nutrients of major concern. They are the driving factors for plant and animal development in most forest stands because they are two of the major building blocks of all organisms.

In most terrestrial environments, nitrogen (N) is found to be the limiting nutrient in the development of trees and other organisms. There are two forms of plant available nitrogen that are commonly found during analysis: nitrate (NO_3^-) and ammonium (NH_4^+). Previous research found that, after harvesting, changes to the forest soil result in an increase in inorganic N (Carmosini et al., 2002; Carmosini et al., 2003; Walley et al., 1996). This can lead to a nitrogen loss through leaching or runoff.

Phosphorus (P) is not commonly a limiting factor in plant growth because of its rapid immobilization by plants and its affinity for being absorbed by clay particles. As a result, mature forest stands have very low levels of mobile and available P (Whitson et al., 2005b). Previous, studies have found that there are rapid increases in phosphate-P after harvesting a forest (Piirainen et al., 2004). A long-term evaluation of forest harvesting has not found a positive correlation between harvesting and P mobility (Macrae et al., 2005; Piirainen et al., 2004).

The objective of this study was to improve the understanding of inorganic N and P availability within forested and post harvest stands over multiple years. This information is important as it can provide baseline information to forestry companies on the effectiveness of their mitigation and preventative measures needed for their environmental impact assessment. It is hypothesized that, after a harvesting event, there will be an increase to in

the inorganic N and P availability for several years post harvest and during forest re-establishment.

3.3 Materials and Methods

3.3.1 Site Description

3.3.1.1 Stand Characteristics

LFH and mineral soils horizons were collected from six forest stands within the southern portion of the Millar Western Forest Management Area (FMA) southwest of Whitecourt, Alberta (Figure 3.3.1). The six stands had similar characteristics before harvesting. The study area was within the boreal plains on clay dominant glacial till parent material. The stands were dominated by lodgepole pine (*Pinus contorta*), but Trembling Aspen (*Populus tremuloides*) and black spruce (*Picea mariana*) were present as well in these stands. The stands had an ecosite description of LF-d, which represents the lower foot hills (LF) while *d* represents the ecosite code for that region (Beckingham et al., 1996). The "d" represents a site dominated by lodgepole pine with a large amount of willows, blueberries, bearberries and hair-cap.

Due to harvesting, the understory vegetations changed which resulted in changes in the stands regional codes. The 3-month-old (Stand 2) and one year old (Stand 3) sites were characterized as a LF-f because the average lodgepole in height was less than 0.6 m and grasses covered approximately 30 to 50% of the area (Table 3.3.1). The 2 (Stand 4) and 3-year-old (Stand 5) stands were classified as LF-j because the lodgepole pine were 0.8 to 1 m in height, there was low shrub and grass cover, and common horsetail was found throughout the site (Beckingham et al., 1996). After harvest many of the characteristics of

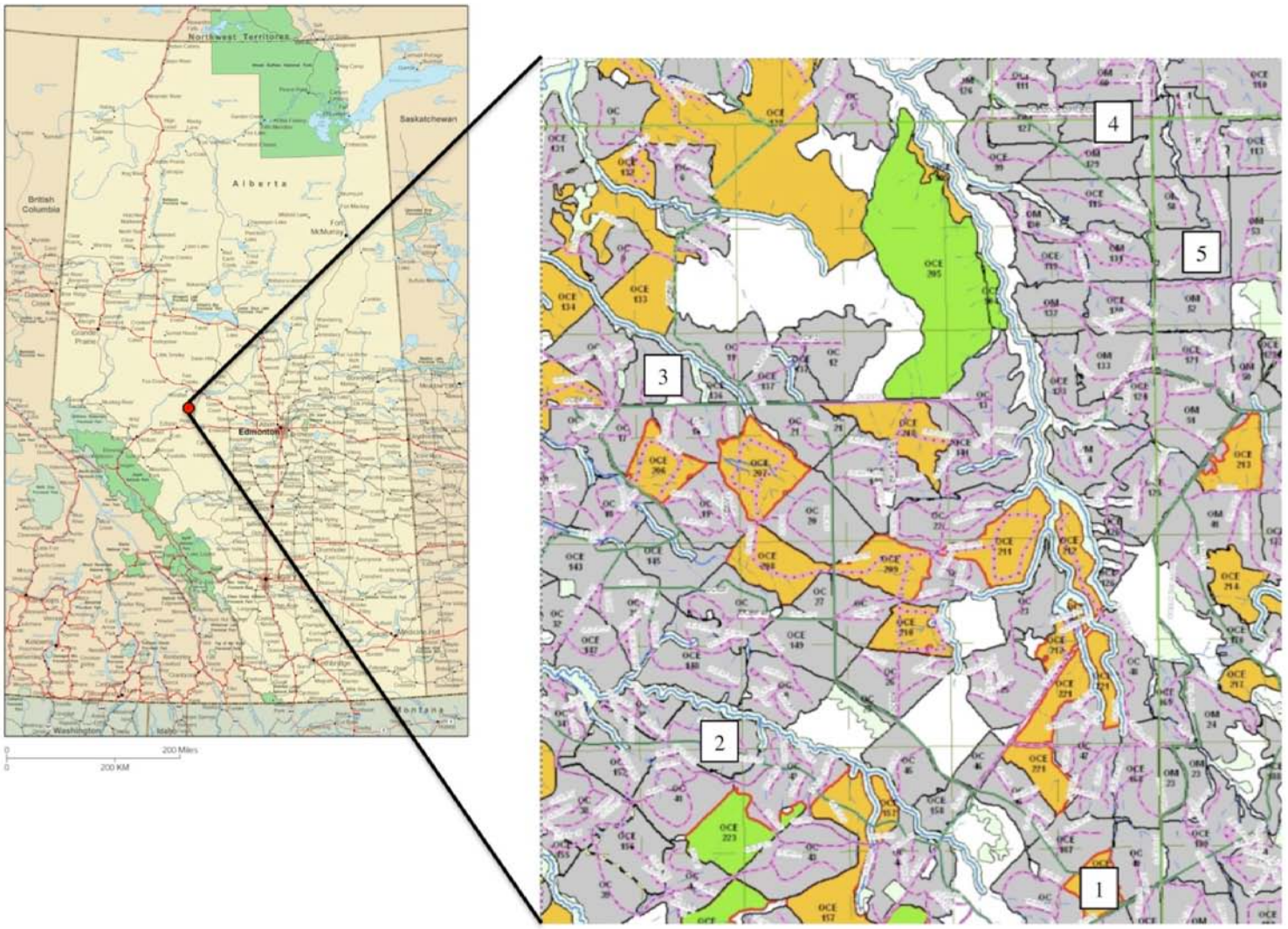

Figure 3.3.1 – This is a chart of west central Alberta and a magnification of the sampling area within the southern portion of the Millar Western FMA. The white boxes with the values 1 to 5 indicate the location of 5 of the six stands sampled during the 2007 and 2008 periods. The numbering of the stands in this study follow the stand designations outlined in Table 3.3.1. The sixth stand was located several km south of sand 1.

Table 3.3.1- Stand characteristics during the 2007 and 2008 sampling seasons. LFH depth and pH were measured during the 2007 season for all six stands.

Stand	Age In May		Slope Aspect	LFH Depth (cm)	Soil pH		Pesticide Application		Trees Present in 2007
	2007	2008			LFH	Mineral	Before or during 2007	2008	
1	~77	0	SW	6.2	5.23	5.37	N/A	NO	YES
2	0.25	1.25	NE	7.7	6.05	6.12	NO	YES	NO
3	1	2	NW	6.7	6.01	6.61	YES	YES	YES
4	2	3	-	7.3	5.60	6.33	YES	NO	YES
5	3	4	-	7.3	5.25	5.77	YES	NO	YES
6	17	18	NE	7.4	6.40	6.47	N/A	NO	YES

the stands remained the same (Figure A.1). The lower areas of each stand contained sphagnum moss. The upper areas contained tall grasses. Three dominant berries were also found on each stand: blueberries, wild raspberries and bearberries. Each stand had some indication of the presence of bears, moose and deer. Another similarity between the stands is that there was a hauling road either adjacent or bisecting the stand.

The soil moisture content was measured for all six stands for the 2007 and 2008 samples (Table 3.3.2). Initial average moisture was low and then increased in the August 2007 samples. The moisture content remained high during the 2008 sampling year (Table 3.3.2). The gravimetric percent moisture was calculated using the soil's wet weight (Wet) and dry weight (Dry) in the following equation (Likens and Bormann, 1999):

$$Percent\_Moisture = \left[\frac{Wet - Dry}{Dry} \right] \times 100$$

3.3.1.2 Soil Characteristics

The soils at each of the sites are Orthic Gray Luvisol as defined by the *Canadian System of Soil Classification* (Soil Classification Working Group, 1998). The soil characteristics were similar to those reported by Whitson *et al.* (2005), with porous LFH horizons that varied from depths of 0 to 16 cm, with mean depths of 6.2 cm in the forested stand and 7.4 cm in harvested stands (Table 3.3.1). The pH of the soils ranged from 5.2 to 6.4 and 5.3 to 6.7 in the LFH and mineral layers, respectively (Table 3.3.1).

When soil sampling took place, the Ae horizons varied in size and were difficult to distinguish between, as a result the soil was divided into two layers, organic and mineral.

Table 3.3.2 – The percent moisture content of soil samples were measured for the six different stands within the Ocelot area of the Millar Western FMA, southwest of Whitecourt Alberta. Nine samples were measured at each site other then stand 6 where 8 samples were measured within the LFH and only 5 samples from the mineral layer. Standard errors were also calculated for each stand (SE).

	2007 June		2007 August		2008 June	
Stands	Mean	SE	Mean	SE	Mean	SE
LFH						
1	79.06	8.03	93.29	4.83	128.52	9.93
2	80.02	15.71	95.64	21.20	141.20	15.11
3	75.35	8.73	99.62	13.45	118.14	13.35
4	77.68	7.02	79.89	6.90	104.38	10.02
5	74.23	7.47	91.06	7.15	115.17	12.25
6	49.46	3.95	156.36	16.21	55.72	11.26
Mineral						
1	23.06	0.63	22.59	0.92	39.03	4.56
2	29.87	2.85	22.89	1.32	35.75	4.25
3	25.61	2.01	27.34	2.28	33.08	3.64
4	23.93	0.85	24.55	1.55	28.55	1.42
5	25.24	1.56	26.51	1.74	31.86	2.26
6	23.19	1.54	20.08	2.21	20.46	1.88

The samples at each stand were taken along the slope at three locations, the upper, mid and foot slope. The upper slopes were upland depressions and contain similar characteristics to that of the foot slopes while the slope angle and aspect changed with each mid slope sample. The mid slopes, slope aspects, are reported in Table 3.3.1

3.3.1.3 Post Harvest Applications

After harvesting, sites were prepared for replanting by the brush, rake and burn method described by Raison (1979). Thus, large debris is raked into a pile within the stands and burned. These piles consist of limbs and tree branches as well as some larger woody debris that was left on the site. Burning, generally, occurs during the late fall, winter or early spring while the area is still wet in order to prevent a forest fire. The sites are then replanted with lodgepole pine seedlings either at the end of the summer after it was harvested or at the beginning of the following summer. To reduce competition for nutrients, herbicides are applied to kill grasses and small vegetation. This generally occurs one or two years after harvesting. Then, additional herbicide applications are done when other vegetation on the stand is competing too much with the planted trees. The years and times of applications are reported for all six stands in Table 3.3.1.

3.3.2 Sampling Design

3.3.2.1 Plot Design

Within each stand, three parallel linear transects approximately 270 m long were established with a minimum buffer of 15 to 20 m from the road to prevent road effects. This was in response to the Radley et al. (2003) study where they suggest that road effects can be found up to 15 m from a forest road. Along the 270 m transect, three sampling areas were selected, approximately 90 m from each other (Figure 3.3.2). At

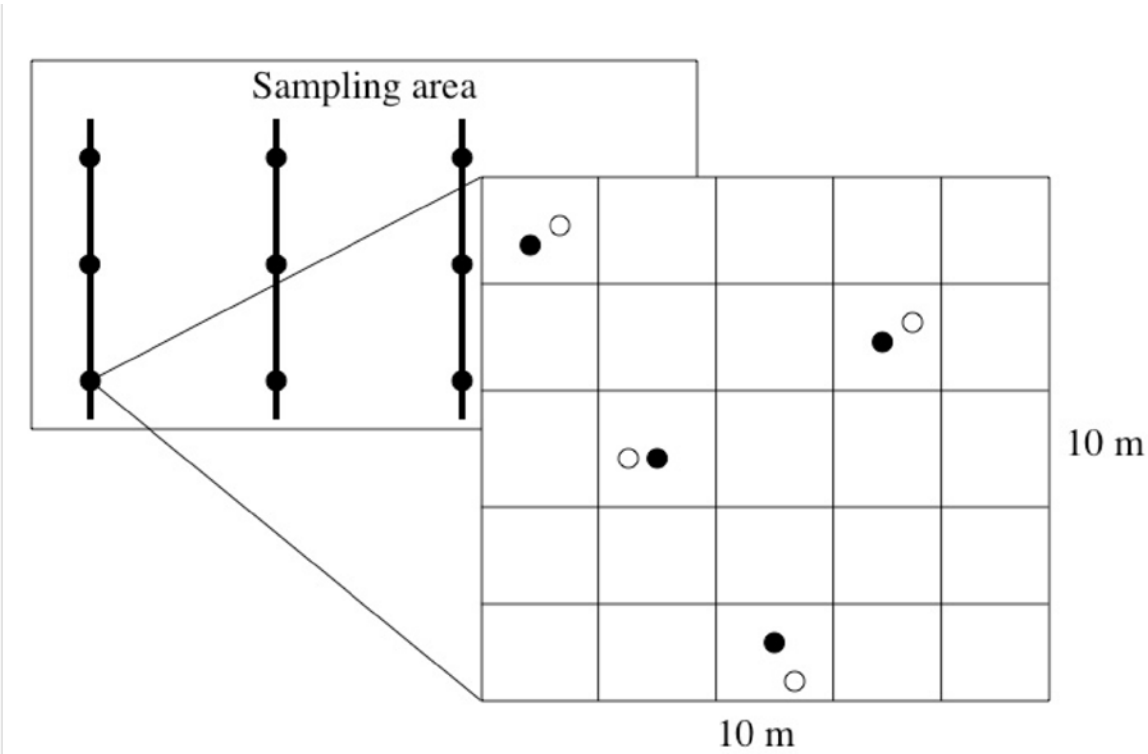

Figure 3.3.2 – A schematic representation of the three transects and three random sampling points along each transect. At each of the three points (closed dot along the transects) a 100 m^2 grid is formed. The four closed circles within the 100 m^2 grid represent the LFH and the four open circles represent the mineral samples. The open and closed circles within the grid represent both nutrient sampling and soil collection for microbial analyses.

each sampling area, a 100 m^2 grid with 25 sampling plots that were 4 m^2 was formed. Four plots were randomly selected through a random draw.

3.3.2.2 Nutrient Sampling

Plant Root Simulator (PRS) probes were used to measure the nutrients available to plants within the sampling area. This PRS probes results are reported as PRS flux and represents nutrient supply rates within the stand (Hangs et al., 2003; Adderley et al., 2006). These are positive or negative charged resin membranes that attract available ions in the soil (Qian and Schoenau, 2002). Two pairs of PRS probes (anion and cation) were placed at each of the four plots. One pair was inserted in the LFH and the second pair was inserted into the mineral layer with a minimal disturbance to the above LFH (Figure A.2). When the LFH layer was very small in depth, the probes were placed at a 45° angle with the resin membrane in the vertical plane. This was done to prevent the resin membranes from suffering loss of nutrients by leaching caused by sub-surface water movement. The probes were removed after six weeks, following the recommendations of the manufacturer as well as previous research (Western Ag Innovations Inc., 2004). A second set of probes was buried at the same location for an additional six weeks.

When the PRS probes were removed, any visible soil particles were wiped off and the probes were stored at 4°C for transport. In the lab, they were rinsed with deionized water, and all four pairs of PRS probes within the same point along each transect and horizon were grouped together and placed in a Ziplock bag. This creates a composite sample for each point along the transect. These samples were then sent to Western Ag for analysis.

PRS probe measurements were only conducted for five of the six stands (Stands 1 to 5) within this study. This was due to a logistical error in not ordering an adequate

number of probes for all six stands. This resulted in the removal of Stand 6 from the nutrient study.

3.3.3 Nutrients

3.3.3.1 Nutrient extraction

Nutrients are eluted from the PRS probes by placing the 4 pairs of probes for each area into a Ziplock bag and 140 ml of 0.5N of HCl were added to the bag (Western Ag Innovations Inc., 2004). The bag is then sealed by first removing all the air from the bag in order to allow the probes to be completely immersed in the HCl solution. The samples were left for 1 hour to allow the eluting to take place. Then, the solution was poured into a clean and labeled dram vial for analysis. In addition, blank probes were also eluted and used as a baseline ion measurement (Western Ag Innovations Inc., 2004).

3.3.3.2 Nutrient Analysis

Ammonium (NH_4^+) and nitrate (NO_3^-) were the two forms of nitrogen measured using the PRS probes. For this analysis, the eluates were run on a Technicon Auto Analyzer II Segmented Flow, which consists of a Technicon AutoAnalyzer II S.C. Colourimeter and Labtronic N.A.P. Software v2.50 (Western Ag Innovation Inc., 2004) for the colorimetric determination of Nitrogen. The detection limits for both NO_3^- and NH_4^+ were 0.1 µg ml^{-1} (Western Ag Innovation Inc., 2004). Total mineral nitrogen was calculated as the sum of extracted mass of NO_3^- and NH_4^+ (product of measured concentration and volume of sample). The nutrient flux to the PRS probe (termed the PRS flux) was determined by dividing the extracted mass by the surface area of the probe.

Phosphorus (P) and other cations and anions (e.g. Ca, Fe, B, K, Mg, Cu, Al, S, Mn, Zn and Pb) in the eluate were measured using inductively coupled plasma (ICP) analysis.

The detection limits varied for each of the chemicals measured. The phosphorus PRS flux was the measurement of phosphate adsorbed on each probe. The phosphorus detection limit for the ICP used by Western Ag Innovation Inc. is 0.01 $\mu g \cdot ml^{-1}$ (2004). The additional cations and anions data can be found in Appendix A (Table A.1).

3.3.4 Statistical Analysis

The statistical analysis used a p-value of 0.1 rather then 0.05 because within forested systems, there are a large amount of environmental and micro-site effects on the sampling points (Zar, 1999). The increase in the p-value will reduce the type II error in these analyses (Zar, 1999). The experiment was structured in a repeated measurement setup with two simple contrast (year, and horizons) and one repeated contrast, which was the nutrient being assessed at each site i.e., ammonium, nitrate or phosphorus. Before the statistical analysis, the data sets were examined for sphericity, which is a means to determine the data's homogeneity. To meet sphericity and to satisfy the homogeneity of the data set ($p > 0.05$), a Greenhouse-Geisser p-value was used in situations where normal sphericity could not be met (Field, 2005). If the data sets are not homogeneous; then a repeated measure analysis of variance cannot be run. Once sphericity was met, the repeated measure ANOVA was used to examine the relationships between stands for both nitrogen and phosphorus. A three-factor design with interactions and repeated measure ANOVA was used to assess the differences between pre and post harvest stand nutrient flux over the 2007 and 2008 sampling periods (Field, 2005). In addition, a secondary multivariate analysis of variance was done on each of the mineral and LFH samples for both years to determine the differences between each site within the same year. A tukey's test was done to indicate the sites that were significantly different between specific

horizons that year. All statistical analyses for this experiment were conducted using SPSS 16.0 software.

3.4 Results

3.4.1 Nitrogen PRS flux

The results of the statistical analysis are presented in Table 3.4.1. Ammonium PRS flux measurements were different between the LFH and the mineral layers of five forest stands with a p-value well below 0.01. The nitrate PRS flux, however, remained the same in both layers of soil ($p = 0.177$). When examining the data more closely, it clearly showed a stand-horizon interaction in the nitrate and ammonium PRS flux, between the two sampling periods 2007 and 2008, with p-values of 0.045 and 0.003 respectively (Table 3.4.1). Meaning that differences between stands were only observed when data were separated by horizons. This indicates that ammonium levels varied based on the different stands. In Figure 3.4.1, one can see a gradual increase in NH_4^+ over 2 years and a rapid decrease of NH_4^+ PRS flux after the first year of harvesting.

LFH and mineral samples were analyzed separately because they were significantly different from each other between years. This was because a repeat measure ANOVA that showed the horizons were different ($p < 0.001$). Annual variations of NH_4^+ PRS flux in the LFH were also significantly different between years ($p = 0.069$). The stands were found to be significantly ($p = 0.003$) different from one another, however there was no effect due to harvest in the NH_4^+ PRS flux ($p = 0.317$). The ammonium within the mineral layer had a similar trend to that of the LFH. The mineral layer, though, was additionally influenced by annual variations in NH_4^+ with a p-value of 0.067. The

Table 3.4.1 - Ammonium, nitrate and phosphorus repeated measure ANOVA results for Homogeneity of the data set and the significant differences between measurements.

	Sphericity [a]		Bonferroni	
Ammonium	df	p-value	F	p-value
Year	1	1.000	0.719	0.421
Horizon	1	1.000	469.228	0.000*
Site	8	0.477	10.030	0.002*
Year x Horizon	3	1.000	17.178	0.003*
Year x Site	10	0.588	1.504	0.248
Horizon x Site	10	0.445	8.709	0.004*
Year x Horizon x Site	11	0.550	1.622	0.225
Nitrate	df	p-value	F	p-value
Year	1	1.000	0.188	0.676
Horizon	1	1.000	2.188	0.177
Site	8	0.123	23.029	0.001*
Year x Horizon	3	1.000	5.627	0.045*
Year x Site	10	0.043	4.182	0.075*
Horizon x Site	10	0.556	14.269	0.005*
Year x Horizon x Site	11	0.062	1.905	0.205
Phosphorus	df	p-value	F	p-value
Year	1	1.000	0.419	0.536
Horizon	1	1.000	1119	0.000*
Site	8	0.196	1.149	0.352
Year x Horizon	3	1.000	0.490	0.504
Year x Site	10	0.405	17.313	0.000*
Horizon x Site	10	0.243	1.333	0.279
Year x Horizon x Site	11	0.244	5.891	0.001*

*- Indicate the significant differences between variables measured. alpha = 0.1
a - Sphericity must have a p-value > 0.05 to accept the assumptions of a repeated measure ANOVA

relationship between the sites was found to be significantly different ($p < 0.001$). When taking these two variables into account within the statistical analysis, the relationship between site relative to year were found not to be statistically significant with a p-value of 0.128 (Figure 3.4.1).

Nitrate (NO_3^-) data was not significantly different between the horizons but there was a difference between years and horizons ($p = 0.045$), similar to that of the NH_4^+. This resulted in the separation of data in year and horizons. When this was done the data was then compared to determine if there was any interactions between stands or within the stands. The statistical analysis indicated that there was no difference between stands ($p > 0.4$).

3.4.2 Phosphorus PRS Flux

The phosphorus PRS flux data was analyzed using the same method as the nitrogen flux. The repeated measure ANOVA for phosphorus indicated that the LFH and mineral layers were significantly different from one another ($p < 0.001$). Even though there was a significant difference between the layers, there was no significant difference ($p = 0.504$) between years (Table 3.4.1). The two years have similar phosphorus PRS flux for each soil layer.

There is a clear relationship between harvesting and phosphorus PRS flux. There was a dramatic increase in phosphorus PRS flux within the LFH and mineral layers once the mature stand was harvested in 2008 (Figure 3.4.2). The phosphorus PRS flux in the LFH after harvest was extremely high, at 120 $\mu g \cdot cm^{-2} \cdot (12weeks)^{-1}$, whereas within the mineral layer there was a smaller increase. Furthermore these increases were significantly different when compared to the 2007 data (p-values of 0.005 in LFH and 0.020 in mineral

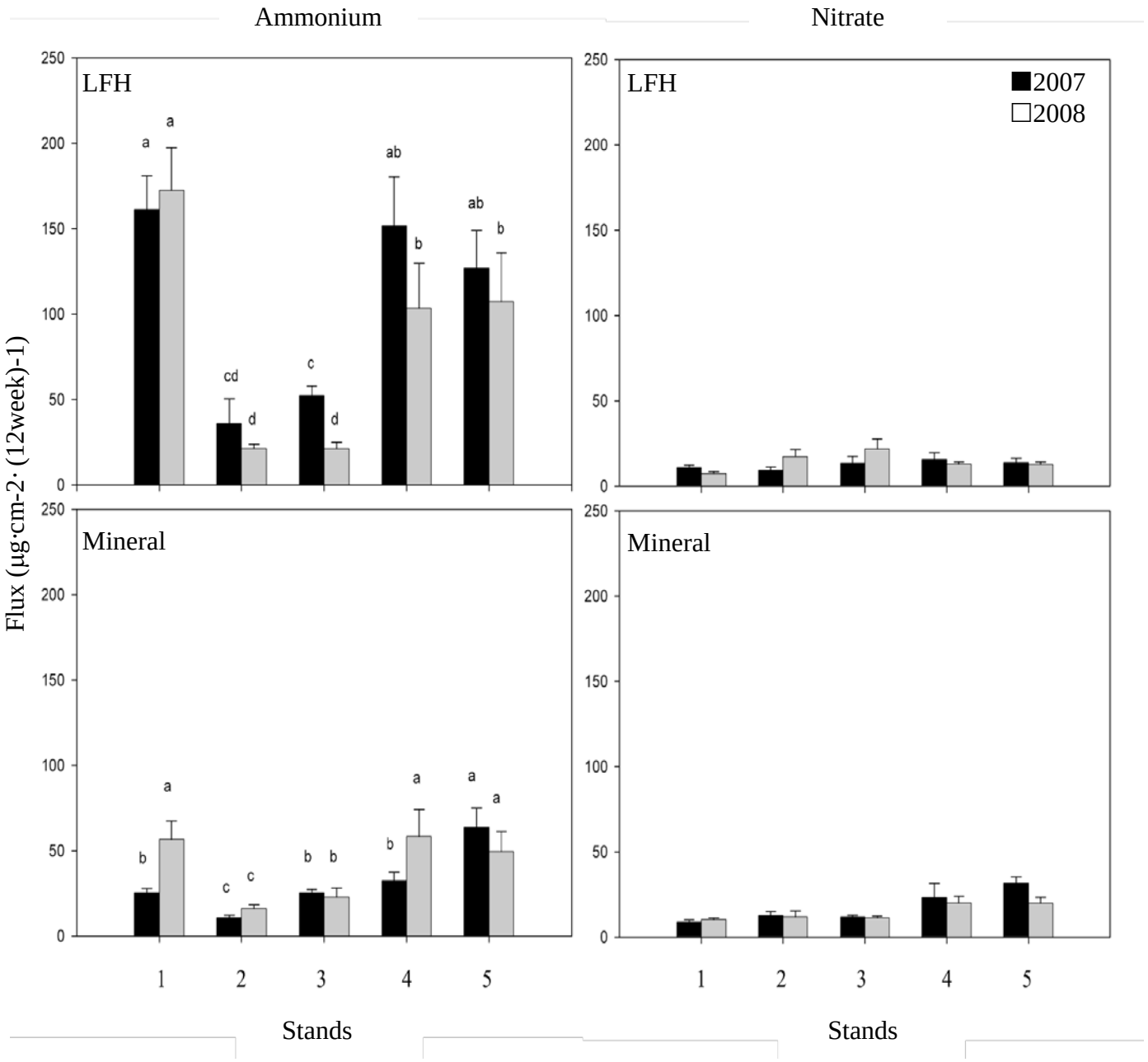

Figure 3.4.1 - Nitrogen PRS flux measurements, in the form of ammonium and nitrate, within the LFH and mineral layers during the 2007 and 2008 sampling periods. Five different stands were examined for similarities and differences. The lower case letters above the bars indicate statistical differences between the samples and sampling period

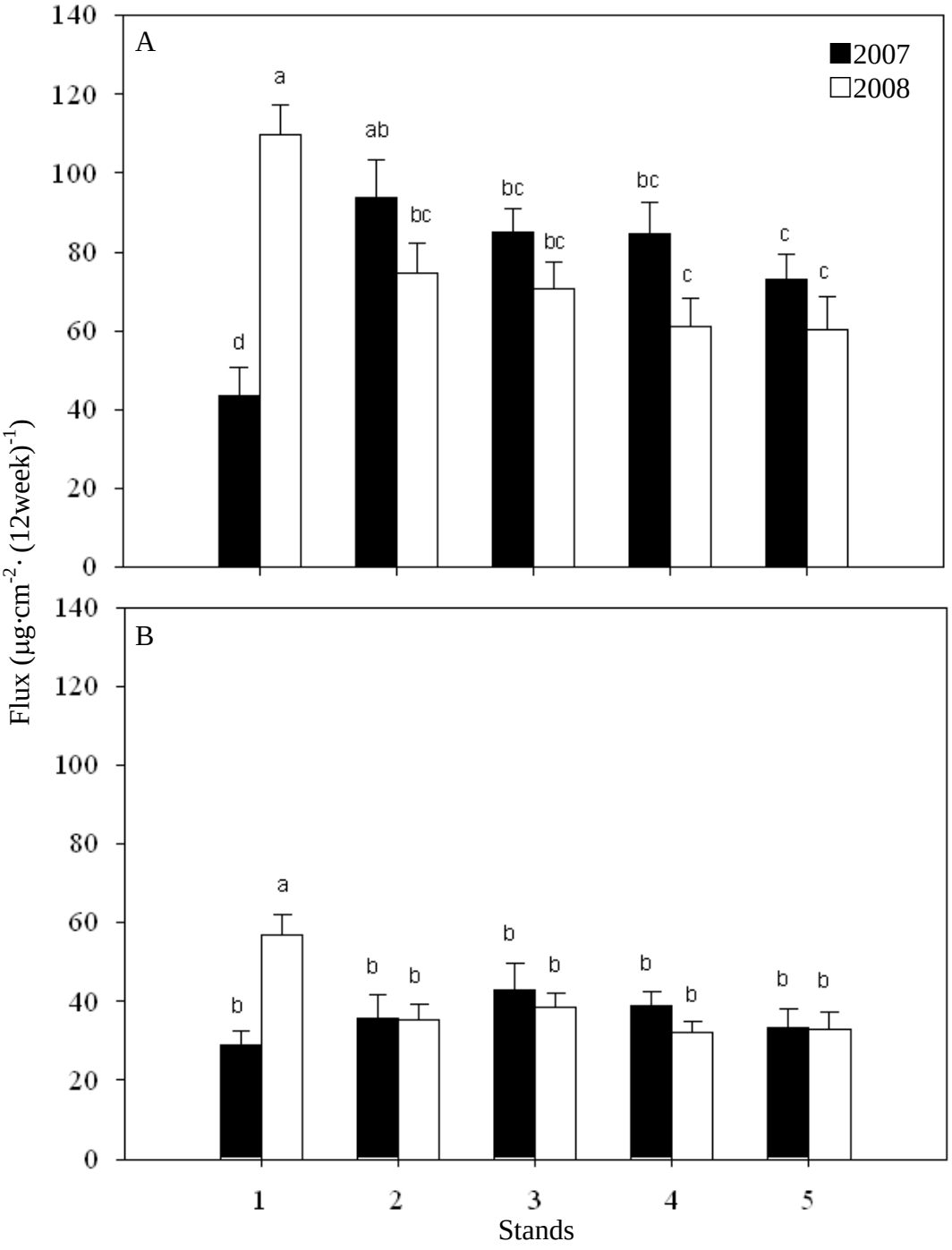

Figure 3.4.2 – Phosphorus flux measurements of the LFH and mineral layers during the 2007 and 2008 sampling period of June to August within west-central Alberta. The lower case letters above the bars indicate statistical differences between the samples and sampling period. The stand descriptions are provided in table 3.3.2.

layer). The phosphorus PRS flux of the recently cut stand exceeded that of all other post harvest stands. As more time passed after harvest, there was a decrease in the PRS flux of phosphorus within the LFH, and mineral layer which then stabilized. In this study, it took approximately three years for the phosphorus levels in the mineral layers to stabilize.

3.5 Discussion

Nitrogen and phosphorus PRS flux measurements were taken over a two-year period (2007 and 2008) in five different stands in order to investigate the influence forest harvesting had on nutrient availability.

3.5.1 Availability of ammonium and nitrate

A large amount of logging residue was left within the stands following harvesting. It was believed that these residues would result in an increase in nitrate and ammonium availability within the stand (Palviainen et al., 2004). From the results collected during the 2007 and 2008 sampling years, the harvested stands did not demonstrate statistically significant changes in nitrate and ammonium PRS flux. However, previous studies examining nitrate and ammonium show increases in their availability. The major increase was, generally, observed in soil nitrate levels because it accompanies the base cations and easily dissolved into the soil solution (Likens et al. 1969; Dahlgren and Driscoll 1994; Homann et al. 1994; Titus et al. 1997). This forest system under study lacked the ability for this to occur due to its low N concentration. This may be resulting from the soil's C to N ratio because an increase in C causes the immobilization of N (Stottlemyer et al. 1995; Maynard 1997). However, in my study the lodgepole pine was very different than the tree species (e.g. sugar maples, yellow birch, red alders and balsam fir) in those studies where

nitrate increased.

Our results then coincide with the Carmosini et al. (2003) study, which examined the mineralization and nitrification in trembling aspen forest soils on the boreal plain. There are several potential reasons why changes in N leaching were not observed. Minimizing soil disturbance from logging during the winter months does contribute to reduced N loss. In addition, a cool and a short growing season allows for minimal change in soil temperature and moisture, which results in a reduced change in mineralization and nitrification, as outlined by Carmosini et al. (2003). The minimal change in available N after harvesting is not specific to the lodgepole pine stands in west central Alberta. The rapid turnover with minimal change to N availability also occurs in the Boreal Shield (Westbrook et al., 2000).

The absence of a change in available N within the forest soil system does not indicate that nitrate and ammonium are not being made available. Rather, it shows that immobilization of these nutrients is very high and masks the total amount of mineralization taking place (Carmosini et al. 2003). This may be caused by the reduced impact of forest harvesting on the plants and microbes within the stand. The minimal impact of the plants and microbes allow them to rapidly immobilize the mineralized N.

3.5.2 Availability of phosphorus

Phosphorus is a very immobile element within forested systems, as it is generally found at low concentrations within the soil solution (Peaslee and Phillips 1981). My study demonstrated an increase in P PRS flux within the forest soil. It showed that there is a large increase in P PRS flux within the LFH horizon after harvesting, which gradually decreases over time. My data collection from stands up to 4 years of age for the LFH

shows that P PRS flux has not decreased to levels as low as that found in a mature stand.

The rapid increase in the availability of P after harvesting can be due to logging residue left on the stand. Phosphorus is rapidly released from the logging residue through a mechanical leaching and decomposition (Palviainen et al. 2004). In addition, decomposition rates increase because of increased soil temperature and moisture, which results from the loss of above ground biomass after harvest (Arnold et al., 1999).

The mineral layer, however, had only a minor increase in P PRS flux after the stand had been harvested and, a year after harvest, returned to levels similar to the mature stand. The lack of change within the mineral layer may have been due to the clay soil, magnesium (Mg), calcium (Ca), aluminum (Al) or iron (Fe) present within this layer (Tate and Salcedo 1988; Gobran et al. 1998). The levels of clay, Mg, Ca, Al or Fe in the soil can cause P to form insoluble complexes, which allows for an observable reduced change in P PRS flux. Any increase in P availability within the mineral layer after cutting may not be from the mobilization of P within the mineral layer, but rather from the leaching of P from the LFH into the mineral horizon. Whitson et al. (2005b) have suggested that this could occur, especially in Orthic Grey Luvisols because it is montmorillonite dominant and sorbs less P.

3.6 Conclusion

To conclude, changes within the nutrient availability were isolated to phosphorus. Nitrogen in the form of nitrate did not show any change as the result of harvesting or re-growth within post harvest stands. Ammonium did show an increase within the harvested stand. There is, however, a potential masking of the mineralization of nitrogen, as nitrogen may be rapidly taken up and immobilized by vegetation and other

microorganism. In addition, there is insufficient data to discount a seasonal environmental effect. Phosphorus availability showed a rapid increase after harvesting and then it gradually decreased over time.

My study examined nitrogen and phosphorus flux to PRS probes. To better understand the nutrient flux and cycles within the stands it would be important to also measure the total and organic forms of nitrogen, phosphorus and carbon present in the soil. This would help provide a better understanding of the nitrogen and phosphorus flux in relation to what is stored within the system.

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CHAPTER 4
Microbial Community Structure within pre and post harvest Stands of Lodgepole pine in West Central Alberta

4.1 Preface

Sustainable forest management relies on the ability of a stand to maintain a steady state in soil characteristics, like pH, soil structure, nutrients, and microbial communities, throughout harvesting and the re-establishment of a tree population. Harvesting can be the cause for changes happening within the microbial community structure, which can impact the movement of nutrients. This can lead to the inability of a forest population to re-establish itself on a stand. This study examines six forest stands at different points of harvest and re-growth, to determine how the microbial community structures react to current harvesting practices.

4.2 Introduction

Bacteria and fungi are two major groups of microorganisms that play a critical role in the biogeochemical cycle in the soil (Allen and Schlesinger, 2004). It has been well documented that several types of these microbes, like different species of fungi, or bacteria, convert complex carbon chains like proteins, complex sugars and starches into their fundamental forms. These organisms, generally, make a wide variety of nutrients available (e.g. carbon dioxide, nitrate, ammonium and phosphate) through decomposition and degradation. Microorganisms are considered to be ubiquitous, but affected by abiotic

variables such as pH, EC, moisture, temperature etc. (Waldrop et al., 2000). The use of biochemical and molecular techniques allows functional and physiological characteristics of microbial communities to be determined. This provides a better understanding of their role in ecosystems.

Soil quality and quantity have been linked to the structure and function of heterotrophic soil microbial communities within forest soils (White et al., 2005). Once forest harvesting takes place, in the form of clear-cutting, changes to the physical characteristics of the soil occur. These changes influence the structural and functional characteristics of the soil microbial communities. The change to the microbial community can be seen as a feed back loop, altering the labile pool of nutrients and manipulating its own role in nutrient conservation within the soil (Radley et al., 2003).

The objective of this study is to examine pre and post harvest forest stands' microbial communities using community level physiological profile (CLPP) analysis and phospholipids fatty acid (PLFA) analysis. This will help determine if there is a long term change in the microbial community structure within post harvest stands as well as when the microbial community reaches a structure similar to that of the pre harvest stand.

4.3 Materials and Methods

4.3.1 Site Description

This study was a multiple field study with 6-forested lodgepole pine dominant stands in the Millar Western FMA during the summer of 2007 and 2008. Treatments included one mature stand (~77 yrs) and five post harvest stands, ages 0.25, 1, 2, 3 and 17 yrs. Each had similar soil profiles and pre harvest characteristics as described in Section 3.3.1. In addition, Table 3.3.1 provides specific information about the stands.

4.3.2 Sampling Design

4.3.2.1 Plot Design

Within each stand, three parallel linear transects approximately 270 meters long were formed with a minimum buffer from the road of 15 to 20 meters to prevent road effects. This was in response to the Radley et al. (1996) study that suggests that road effects can be found up to 15 meters from a forest road. Along the 270 m transect, three sampling areas were selected, approximately 90 meters from each other. At each sampling area, a 100 m^2 grid with 25 sampling plots and $4m^2$ wide was formed (Figure 3.3.1). Four plots were randomly selected through a random draw.

4.3.2.2 Soil Sampling

Samples were collected three times, once at the end of May, once in mid July and again at the end of August. These were taken from the same plot where the PRS probes were planted. Transects were placed parallel to one another on a sloping surface. Four samples from different plots within the 100 m^2 area at each point along these transects were bulked together to form one composite sample of the point. Samples were taken using a 1 ½ inch back saver probe (Figure A.3). The LFH and 10 cm of the mineral layers were separated using a soil knife and stored separately. Once samples had been taken, a 1 m^2 area within each stand was used to estimate the percent vegetation cover and the dominant plant species were identified (Table 3.3.1). This allowed us to provide each site with an ecosite code as described by Beckingham et al. (1996). Samples were stored in a whorl-pak bag and at a temperature of approximately 4 °C for transportation. After these transported samples were sieved with a 2 mm mesh, sub-sampled and stored at 4 °C for

CLPP and at -20°C for molecular techniques (1998). Remaining samples were stored for long-term storage at -20 °C.

4.3.3 Community level physiological profiles (CLPP) Analysis

Microbial communities within the soil were assessed for 32 different carbon substrates using Biolog ecoplates (Table 4.3.1). 31 carbon substrates can be grouped together into six major categories called guilds, which determined the CLPP of the six different stands within this study. The CLPP was done in accordance with the method outlined by Siciliano and Germida (1998) with some amendments. An increase to 150 µl of soil slurry inoculums occurred, following the recommendations by Biolog Inc. (2002), while the soil slurry dilution was increased to a 10^{-5} dilution. Briefly, the soil slurry was made using 5 g of field moistened soil. The soil was placed in a nalgene bottle with 45 ml of sterile phosphate buffer solution (PBS) at a pH of 7.6 and shaken for 20 minutes at 200 rpm. One ml of this solution was removed and placed in a test tube containing a 9ml solution of PBS. This dilution method was repeated until a dilution of 10^{-5} was met. 150 µl of a 10^{-5} dilution of soil slurry was loaded into each well of the ecoplate using a multi channel pipettor.

The Biolog ecoplates were then incubated at 27 °C for 96 hrs. After which time, the plates were removed and their optical densities measured using a microplate reader (Biolog Inc) at a wavelength of 590 nm. The data was collected on an excel file and paper for long-term storage. All wells were divided by the control well and all negative values were reset to 0 (Selmants et al., 2005). To compare between the plates, they were standardized by dividing each well in the plate by the sum of the total plate's

Table 4.3.1 – The substrate list of the 31 different carbon substrates and a control sample that are utilized within a Biolog ecoplate. In addition the chemical guild have been provided for each substrate in accordance to Choi and Dobbs (1999).

Carbon Source	Guilds
water	Control
h-methyl-d-glucoside	Carbohydrate
d-galactonic g-lactone acid	Carbohydrate
l-arginine	Amino acid
Pyruvic acid methyl ester	Carboxylic acid
d-xylose	Carbohydrate
Galacturonic acid	Carboxylic acid
l-asparagine	Amino acid
Tween 40	Polymer
i-erytritol	Carbohydrate
2-hydroxybenzoic acid	Phenolic compound
l-serine	Amino acid
Tween 80	Polymer
d-mannitol	Carbohydrate
4-hydroxybenzoic acid	Phenolic compound
l-phenylalanine	Amino acid
a-cyclodextrine	Polymer
N-acetyl-d-glucosamine	Carbohydrate
g-hydroxybutiric acid	Carboxylic acid
l-threonine	Amino acid
Glycogen	Polymer
d-glucosaminic acid	Carboxylic acid
Itaconic acid	Carboxylic acid
Glycyl-l-glutamic acid	Amino acid
d-cellobiose	Carbohydrate
Glucose-1-phosphate	Carbohydrate
a-ketobutiric acid	Carboxylic acid
Phenylethylamine	Amine
a-d-lactose	Carbohydrate
d-l-a-Glycerol-1-phosphate	Carbohydrate
d-malic acid	Carboxylic acid
Putrescine	Amine

optical density (McCune and Grace, 2002). This allowed us to compare the average well color density (AWCD) between soil samples and stands.

4.3.4 Phospholipid fatty acid (PLFA) analysis

The PLFA analysis enabled us to examine the different groups of microorganisms present within the soil samples. This provided an estimate of the relative biomass of the different populations within the communities. PLFA was done following the modified method of White et al. (1979) described by Helgason et al. (2009) with an additional modification for soils with high organic matter as described by Balser (2001). Briefly, 4.0g of mineral soil and 1.0 g of LFH soil were lyzophilized using a ratio of 2:1:0.8 (v:v:v:) and 6:3:2.4 (v:v:v:) respectively, of MeOH:$CHCl_3$:P buffer. The phospholipids were separated from the glycolipids and neutral lipids in a solid phase extraction (SPE cartridge; Bond Elutes). The phospholipids were then methylated. The resulting fatty acid methyl esters were then run through a Hewlett Packard 5890 Series II gas chromatograph with a 25 m Ultra 2 column (J&W Scientific) for analysis. The sample peaks from the gas chromatograph were then identified using MIDI identification software (MIDI) to designate the specific biomarkers present within each soil sample analyzed. A group of biomarkers was used to characterize the microbial communities within each sample (Table 4.3.2). To standardize the PLFAs, the internal standard (19:0) was used to determine the concentration per gram for each peak within the histogram following the calculation described by Hedrick et al. (2005). The resulting fatty acid measurement, which represented different groups of microbial populations, was then statistically analyzed to examine the relationship between the PLFA fingerprints of each stand.

Table 4.3.2 – Biomarkers that are predominant indicators of forest soil microbial activity as found in literature. These biomarkers have been broken down into types (bacteria, fungi and actinomycetes) and any associated groups (Gronli et al., 2005; Leckie, 2005).

Biomarkers	Type	Group
15:0	Bacteria	N/A
17:0	Bacteria	N/A
16:0	Bacteria	N/A
16:1w8	Bacteria	Methane-oxidizing
18:1w8	Bacteria	N/A
16:1w5t	Bacteria	N/A
16:1w6c	Bacteria	N/A
i17:1	Gram +	Anaerobic
a16:0	Gram +	Anaerobic
i17:0	Gram +	Anaerobic
a14:0	Gram +	Anaerobic
cy19:0	Gram -	Anaerobic
i16:0	Gram +	N/A
i15:0	Gram +	N/A
i14:0	Gram +	N/A
cy17:0	Gram -	N/A
br17:0a	Gram -	N/A
br16:1	Gram -	N/A
br16:0	Gram +	N/A
a17:0	Gram +	N/A
a15:0	Gram +	N/A
18:1w7c	Gram -	N/A
16:1w9c	Gram -	N/A
16:1w7c	Gram -	N/A
16:1w5	Gram -	N/A
16:1	Gram -	N/A
12Me16:0	Gram -	N/A
10Me16:0	Gram +	Anaerobic
18:1	Actinomycetes	N/A
10Me18:0	Actinomycetes	N/A
10Me17:0	Actinomycetes	N/A
20:1 w9c	Fungi	N/A
18:3 w6c (6_9_12)	Fungi	N/A
18:2 w9c	Fungi	N/A
18:2w6	Fungi	N/A
18:3w3	Fungi	N/A
18:1w9c	Fungi	N/A
16:1w5c	Fungi	Anaerobic

4.3.5 Statistical Analysis

The soil microbial community PLFA and CLPP profiles were compared by non-metric multidimensional scaling (NMS) analyses. This method was used instead of PCA because NMS is a robust method of analysis that looks at multiple iterations of the data set to provide the best ordination with minimal constraints. In addition, a Monte Carlo test was done to help determine the dimensions to use. The dissimilarities between the data sets were determined using a Sørensen distance measurement, which is better known as the Bray-Curtis distance measurement (Friedel et al., 2006). To determine the stands relationships, an MRPP and a pairwise comparison was conducted on each NMS data (See Example Appendix B). The community level physiological profile analysis data was then grouped into their guilds and compared using an ANOVA to examine them between years and stands. The phospholipids were grouped into three groups, however; only fungi and bacteria were examined for the physiological community's abundance. An ANOVA was used to examine the statistical differences in microbial communities between the stands.

4.4 Results

4.4.1 Community physiological profile (CLPP)

Using a non-parametric multi-dimensional scaling (NMS) analysis, we examined the relationship between the stands, the LFH and mineral layers. This showed that there were differences in the functional characteristics of the microbial community between the LFH and mineral layers within all six stands (Figure 4.4.1). The functional characteristics of the microbial community within the LFH and mineral layer of all the stands were similar to one another. This relationship was also present in the mineral layers. 67.7 % (NMS1) of the variability between samples was explained through the separation of soil layers. Only 17.6% (NMS2) of the variability was explained between stands. It was apparent that the mature, the freshly cut (0.25) and the 3 yr old stands, differed from each other and that the 17, 1 and 2 yr old stands differed from each other in their LFH. The mature stand's mineral samples were indistinguishable from all the other stands and vice versa. This relationship allowed us to examine the functional microbial communities within the LFH and mineral layers separately.

Some differences existed between the functional guilds of the microbial communities within the pre or post harvest stands. However, none were statistically significant with p-values greater then 0.1. The LFH layers when compared showed some minor variations between the data set (Figure 4.4.2). There was a slight increase in the consumption of the phenolic compounds, amino acids and amines after the mature stand was cut and these continued to increase even after a year post harvest. These changes were only observed within those two stands; however, the changes were small and proved to be insignificant. The mineral layer remained stable within the pre and post harvest

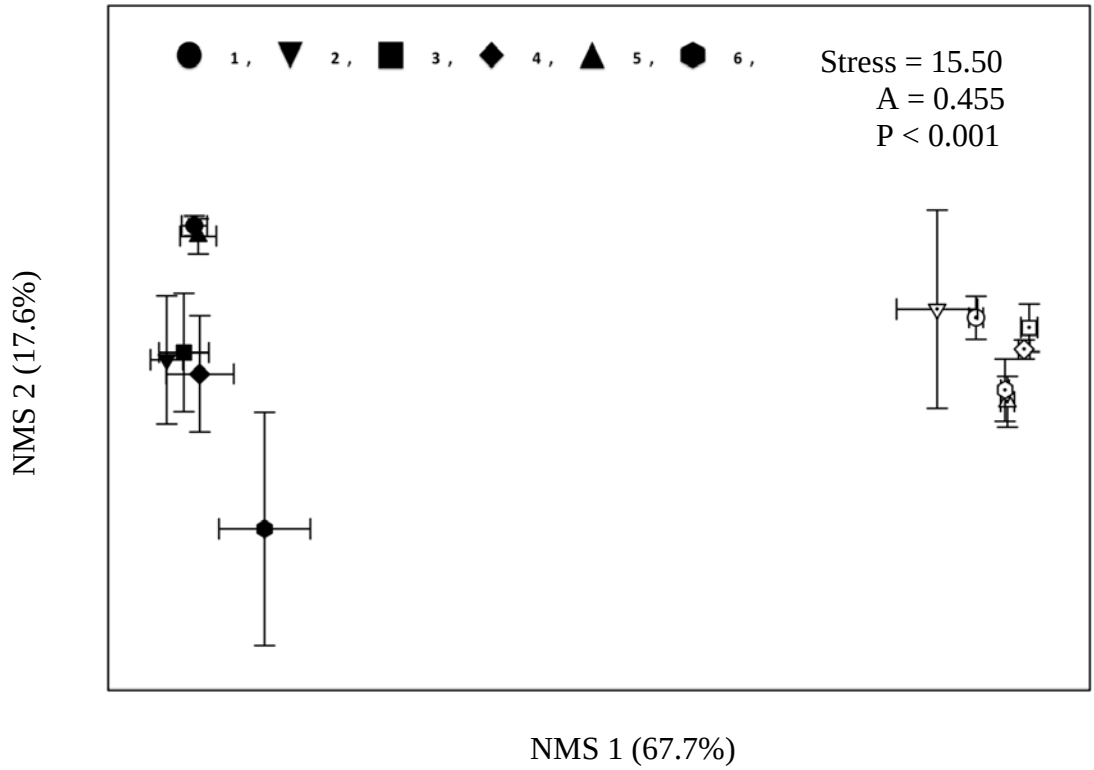

Figure 4.4.1 – NMS of CLPP structure of the LFH and mineral layers obtained from six different lodge-pole pine dominated forest stands of west-central Alberta in 2007. Each symbol is the composite sample of 9 different sampling points within a single stand (n = 9). Closed symbols represent the LFH layers of the stands while the open symbols represent the mineral layer of the stands. Error bars associated with each symbol represents the standard errors of the mean and percent variation are marked in parentheses.

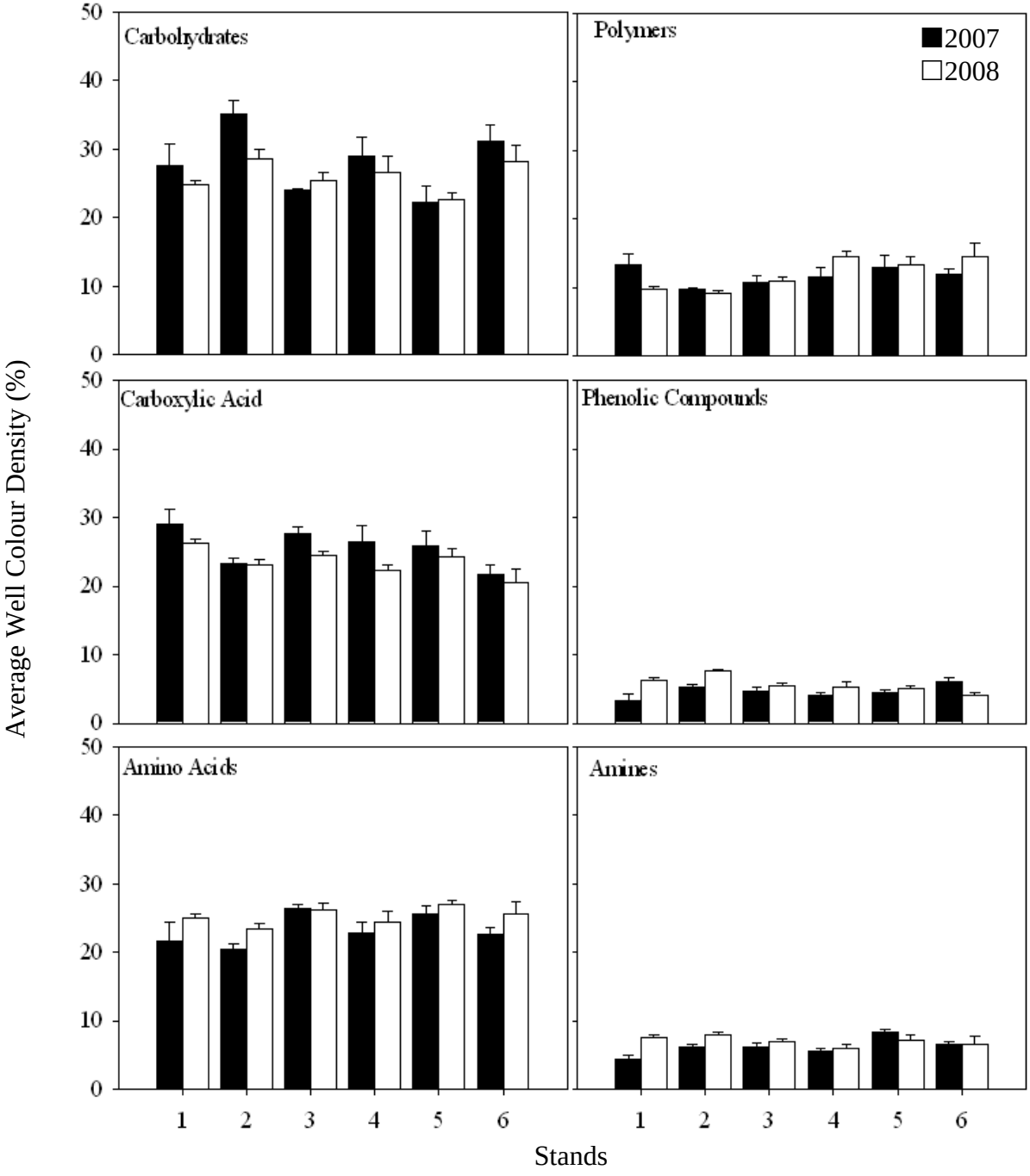

Figure 4.4.2 – The AWCD of the six functional guilds within the LFH layers of lodgepole pine dominant stands of west-central Alberta in the summer of 2007 and 2008. Each bar is a composite of nine sampling points (n = 9) within each stand. The black bars represent the June 2007 period while the grey bars represent the June 2008 period. Error bars for the sample represent the error of the mean for each stand.

stands. After examining 2 years of data of the mineral layer ($p > 0.1$), no significant changes were found in the functional guilds of the stands. After the mature stand was harvested, its microbial community's functional guild compositions within the mineral layer did not deviate from its pre harvest state (Figure 4.4.3).

The non-parametric multi-dimensional scaling (NMS) analysis indicated that carbon substrate utilization by the soil microbial communities associated with LFH changed between June and August samplings within all stands, regardless of the harvesting age (Figure 4.4.4a). At the end of the field season (August), there was a tighter grouping of the stands. While at the beginning of the summer, the mature, 0.25 yr and 3 yr old stands were different from the 1, 2, and 17 yr old stand. This indicates that their microbial communities were different. 83.8% (NMS1) of the variability within this graph explains the difference in functional characteristics of the microbial communities from June and August 2007. However, only 11.1% (NMS2) of the variability was present between stands. Soil microbial communities' utilization of the substrate remained stable in the mineral layer and did not show any significant differences throughout the season (Figure 4.4.4b). When these results were examined, all the stands from the June and August sampling periods were similar to each other with only a slight amount of variability as shown through NMS 1, which shows 44% variability, and the NMS 2 shows 25.8%. One stand seemed distinct from the others. The one-year-old stand stood out in both the June and August sampling periods. However there was ample amount of overlap within the NMS 1 axis that resulted in that sample being similar to the rest of the stands.

When comparing the data collected during June 2007 and June 2008, it was found that the results seemed to be similar to those collected between the June and August in

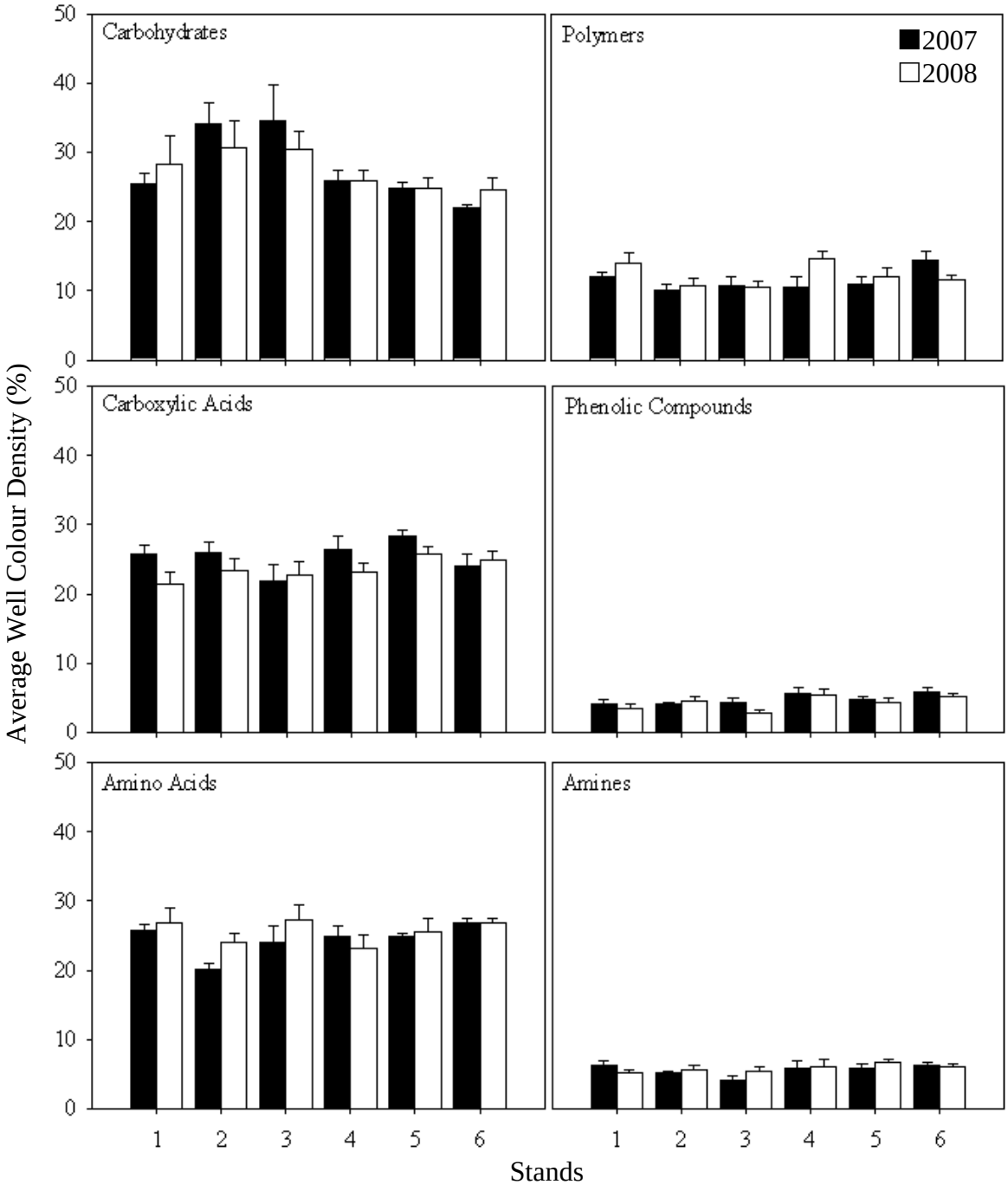

Figure 4.4.3 - The AWCD of the six functional guilds within the Mineral layers of lodge-pole pine dominant stands of west-central Alberta in the summer of 2007 and 2008. Each bar is a composite of nine sampling points (n = 9) within each stand. The black bars represent the June 2007 period while the grey bars represent the June 2008 period. Error bars for the sample represent the error of the mean for each stand.

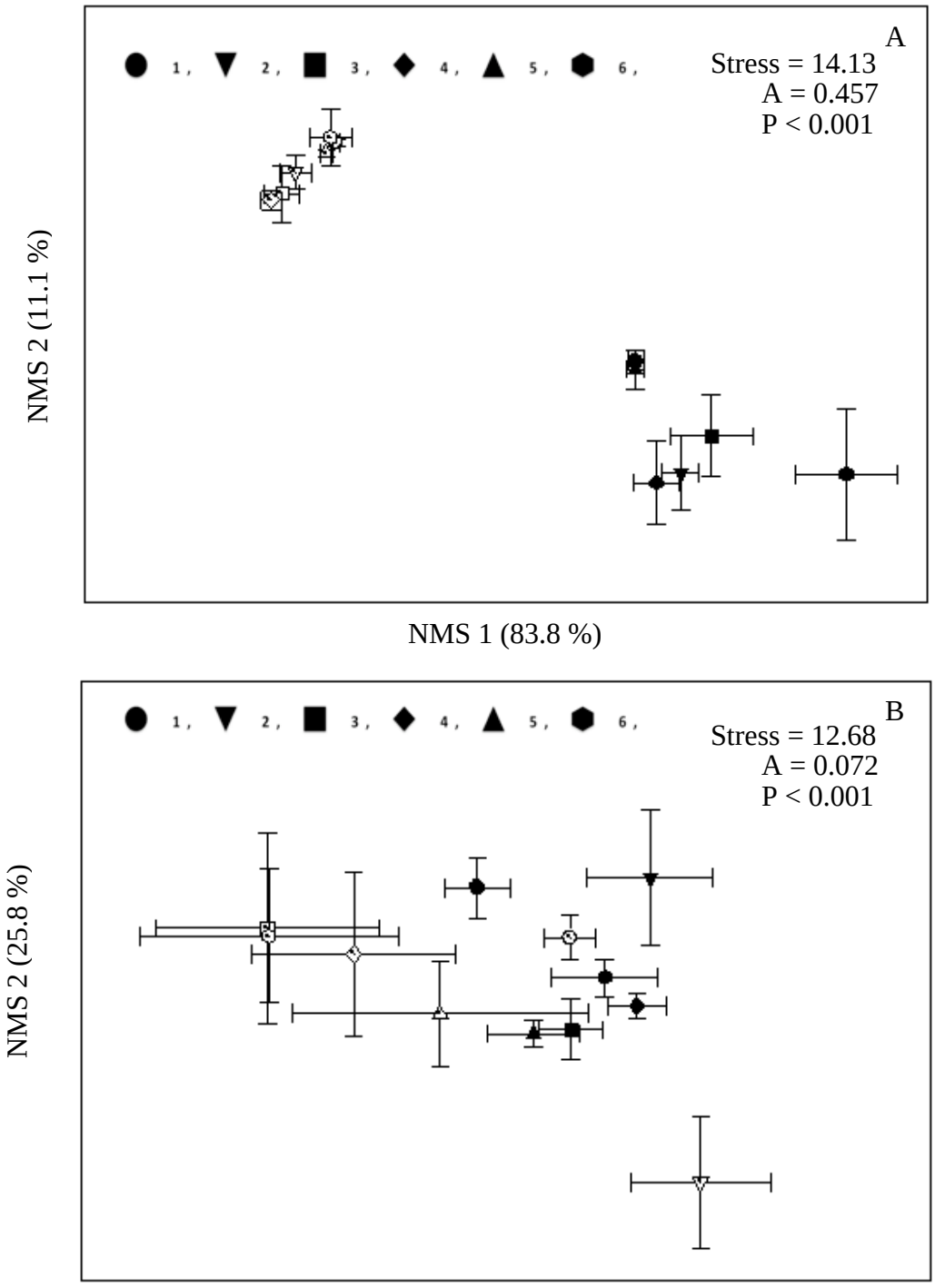

Figure 4.4.4 – NMS of CLPP structure of the LFH (A) and mineral (B) layers obtained from six different lodge-pole pine dominated forest stands of west-central Alberta in 2007. Each symbol is the composite sample of nine sampling points within a single stand (n = 9). Closed symbols represent the June sampling period while the open symbols represent the August sampling period. Error bars associated with each symbol represents the standard errors of the mean and percent variation are marked in parentheses.

Figure 4.4.5a. The LFH samples seemed to separate by year. In 2007, the samples separated out into three groups: the mature, 0.25 and 3yr old stand and, the 1, 2, and 17 yr old stands. In 2008, however, samples seemed to remain together with similar communities. When examining the variability within the data sets, it was found that 74.6% of the variability was between 2007 and 2008 samples. Only 17.6% of the variability represents the difference between the stands. Once again, the mineral samples seem to group together and show no distinction between years (Figure 4.4.5b). There is only a very low variability between stands and year. 28.7% of the variability partially separates the two years, while only 31.8% variability explains the difference between the stands. These together only explain a small amount of variability within the data set.

Due to the large variation between the seasonal and the annual comparison of the LFH, a secondary NMS plot was done looking at the August 2007 and the June 2008 data. Here, a reduced variability between the two years was observed. As on the graph, the two sampling periods appear closer, some separation does exist, but they are rather close and in turn similar to each other. There are larger differences between stands than between sampling periods (Figure 4.4.6). With only 33.0% of the variability related to the sampling period, while 57.7% is related to differences between stands. These changes indicate that an event occurred during the sampling season in 2007 that altered the functional microbial community structure. However, the following year, the microbial community remained the same as that at the end of the previous season.

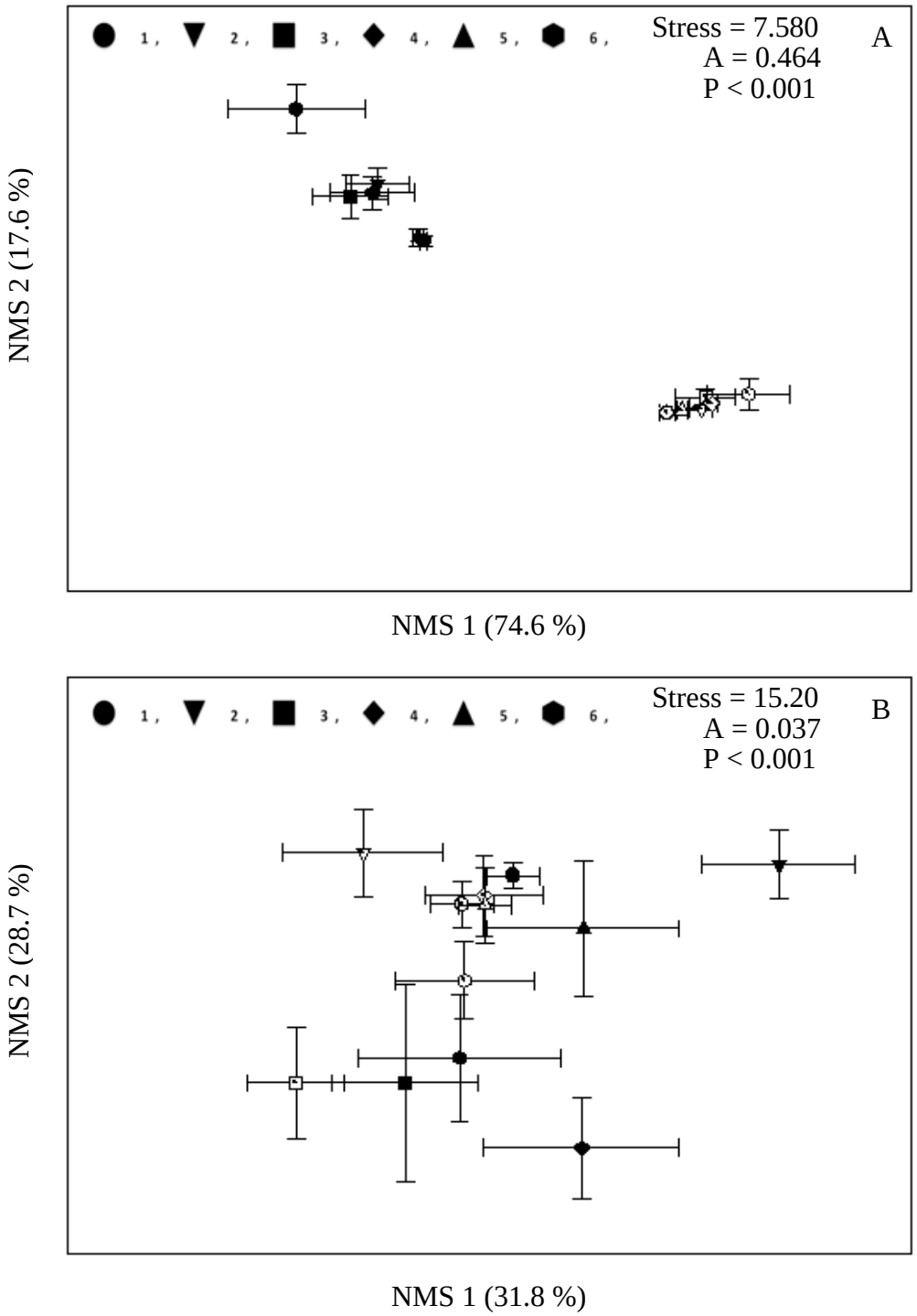

Figure 4.4.5 – NMS of CLPP structure of the LFH (A) and mineral (B) layers obtained from six different lodge-pole pine dominated forest stands of west-central Alberta in 2007 and 2008. Each symbol is the composite sample of nine sampling points within a single stand (n = 9). Closed symbols represent the June 2007 sampling period while the open symbols represent the June 2008 sampling period of the stands. Error bars associated with each symbol represents the standard errors of the mean and percent variation are marked in parentheses.

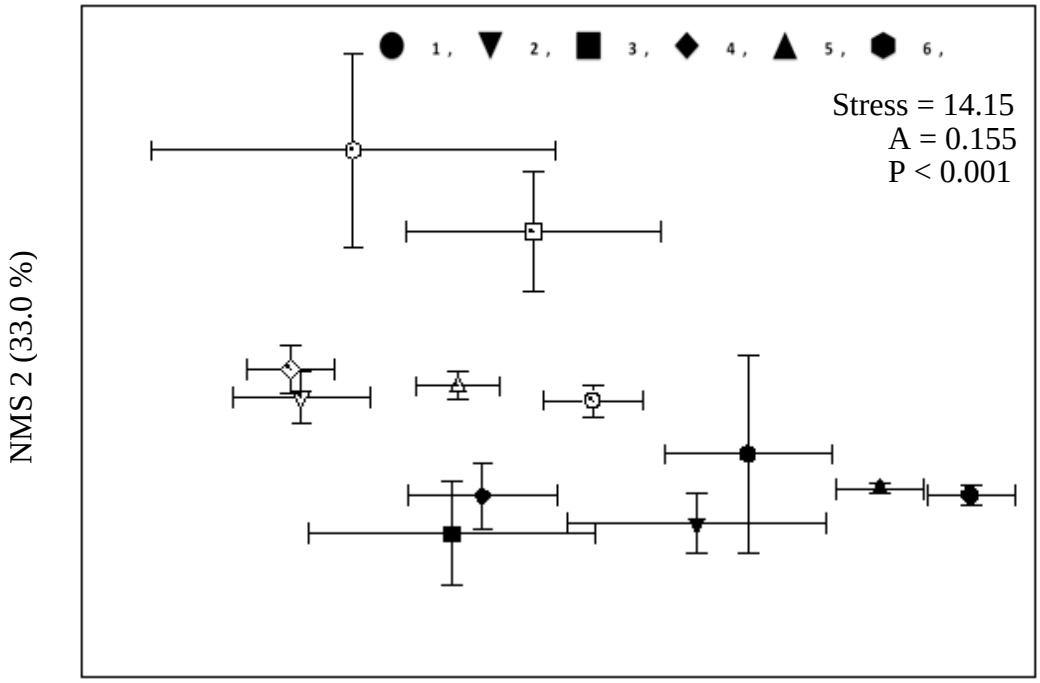

Figure 4.4.6 - NMS of CLPP structure of the LFH layers obtained from six different lodge-pole pine dominated forest stands of west-central Alberta in 2007 and 2008. Each symbol is the composite sample of nine sampling points within a single stand (n = 9). Closed symbols represent the August 2007 sampling period while the open symbols represent the June 2008 sampling period of the stands. Error bars associated with each symbol represents the standard errors of the mean and percent variation are marked in parentheses.

4.3.2 Phospholipid fatty acid (PLFA) analysis

The NMS method was used to determine the phospholipid fatty acid fingerprint relationship to allow an easy comparison between samples. The phospholipid fatty acid (PLFA) fingerprints of the LFH and the mineral layers microbial communities were found to be statistically different. The soil layers were separated along the NMS 1 axis, which represented 65.8% of the variability the layers, while only 33.0% of the variability was found on the NMS 2 axis. When the relationship between the microbial communities within the LFH and mineral layers was examined, the PLFA results showed that they were different communities (Figure 4.4.7). The stands that were studied seemed to be more similar within the same layers than between the different stands. The graph shows that the mineral layer samples group together more tightly than the LFH.

As there is a difference between the LFH and mineral layer samples, we were able to examine the LFH and mineral layers separately. The LFH layers were examined from two different sampling periods during the summer. The first set of samples was collected at the beginning of the sampling period (June), while the second set of samples was collected at the end of the season (August). These results were similar to that of the functional communities. The PLFA fingerprints differ between the two periods (Figure 4.4.8a). When examining the relationship using a pair wise comparison, the two periods differed from each other and the p–value was very low. The results from the mineral layer's sampling periods, June and August, were very similar to that of the LFH. There were two large groupings of samples with the separation based on sampling periods (Figure 4.4.8b). As a result, there is a clear difference between the August and June sampling periods.

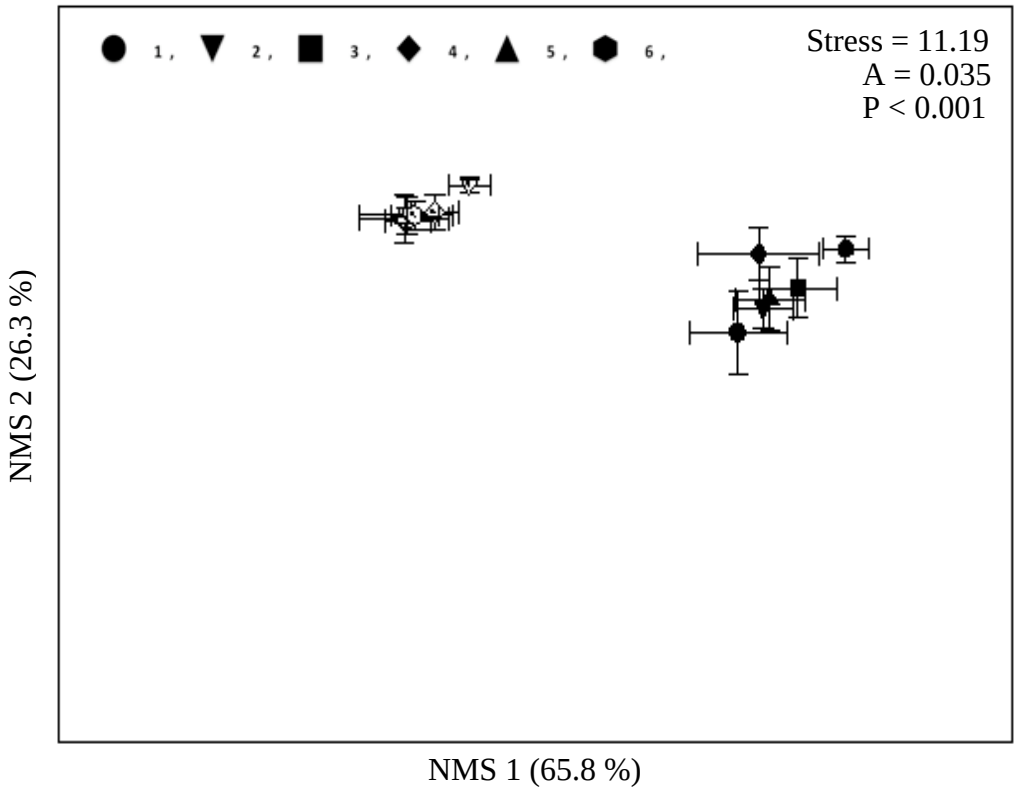

Figure 4.4.7 - NMS of PLFA structure of the LFH and mineral layers obtained from six different lodge-pole pine dominated forest stands of west-central Alberta in 2007. Each symbol is the composite sample of nine sampling points within a single stand (n = 9). Closed symbols represent the LFH layers of the stands while the open symbols represent the mineral layer of the stands. Error bars associated with each symbol represents the standard errors of the mean and percent variation are marked in parentheses.

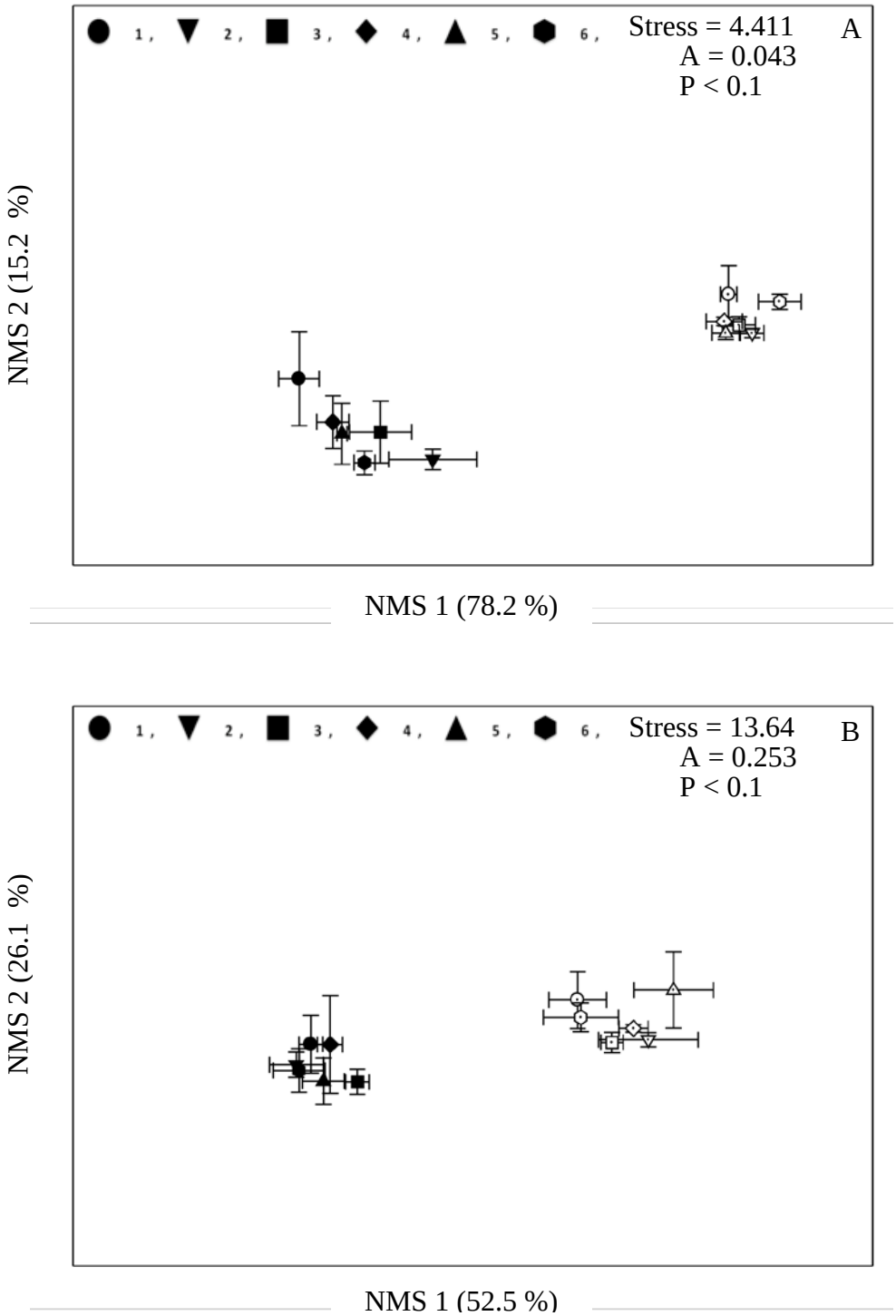

Figure 4.4.8 – NMS of PLFA comparison of the microbial structure of the (A) LFH and (B) mineral layers of the June (closed shapes) and August (open shapes) samples from six different lodge-pole pine dominate d forest stands of West-central Alberta in 2007. Each sample is a composite sample of nine different sampling points within a single stand (n = 9). Error bars for each symbol represent the standard error of the mean and percent variation.

In addition, the microbial communities were analyzed over the two years, 2007 and 2008. As there was a difference between the PLFA fingerprints of the mineral and the LFH layers, we examined the layers separately. No difference occurred between the LFH layer of the June 2007 and June 2008 samples (Figure 4.4.9a). The data showed no difference between the two sampling periods with a very low variability within both axes, NMS 1 and NMS 2, and with a percent variance of 78.7% and 14.3% respectively. However, there were no clear separations between the samples, which indicated no difference in the stands mean community structures. The mineral layer did show a distinct separation between the two sampling years, 2007 and 2008 (Figure 4.4.9b).

As there is a difference between the results obtained from the functional (CLPP) analysis and the PLFA fingerprint, a secondary analysis of the mineral layer PLFA data using the August 2007 and the June 2008 samples was done to determine if there was any difference between these two sampling periods. This was only done for the mineral layer because no differences were found in the LFH PLFA fingerprints. Once again, there was no difference between the two sampling periods, but the NMS 1 and NMS 2 axis have a variance of 23.6% and 67.9% respectively (Figure 4.4.10). This indicates that the community's structures did not change with relation to the effect of moisture as seen in the CLPP data. As there is a large buffering capacity within the LFH layer, the microbial communities remain unchanged in their original structure. However, with a shift in the available nutrients and moisture, the microbes are forced to shift their consumption to secondary forms of nutrients rather than from what was found in the mature stand (Stand 1).

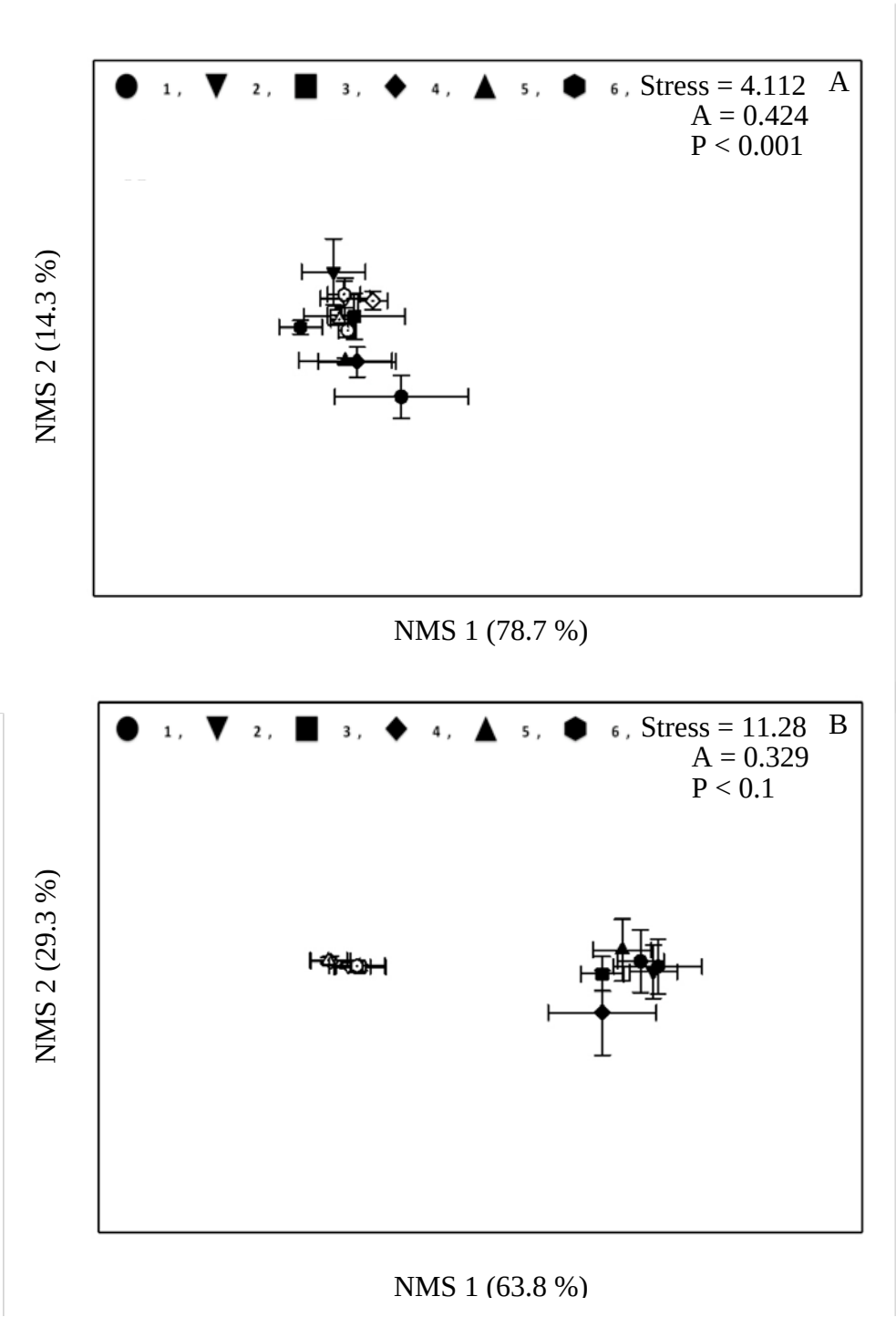

Figure 4.4.9 – NMS of PLFA comparison of the microbial structure of the (A) LFH and (B) mineral layers of the 2007 (closed shapes) and 2008 (open shapes) samples from six different lodge-pole pine dominate d forest stands of West-central Alberta during the beginning of the sampling season. Each sample is a composite sample of nine different sampling points within a single stand (n = 9). Error bars for each symbol represent the standard error of the mean and percent variation.

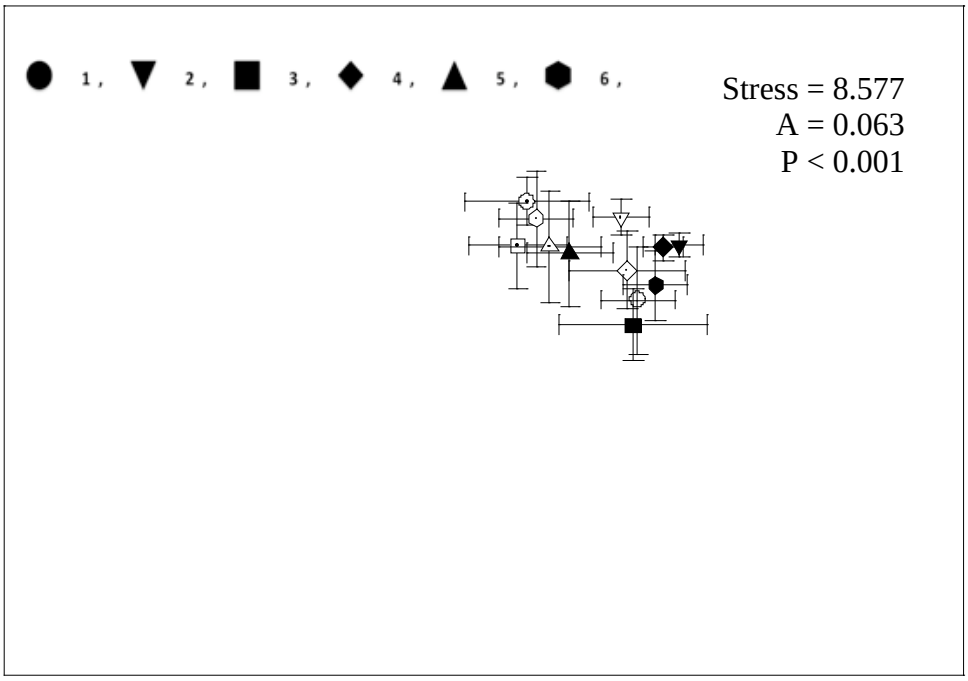

Figure 4.4.10 - NMS of PLFA comparison of the microbial structure of thee mineral layers of the August 2007 (closed shapes) and June 2008 (open shapes) samples from six different lodge-pole pine dominate d forest stands of West-central Alberta. Each sample is a composite sample of nine different sampling points within a single stand (n = 9). Error bars for each symbol represent the standard error of the mean and percent variation.

In addition a secondary means of measuring the change within the samples were done. This was to examine the bacterial and fungal populations and how there ratios change between the two sampling periods. There was a decrease in both the bacteria and fungal populations between the 2007 and 2008 sampling periods (Figure 4.4.11; Figure 4.4.12). This was however only found within the mineral layer while in the LFH the populations remained constant. This was a clear indication that there may be some change occurring between the years. However, when the bacterial- fungal ratio was compared there was little difference between their ratios (Figure 4.4.13). The bacteria populations within stands 4, 5, and 6, visually have a higher level of bacteria-fungal ratio, however statistically the 2008 samples are not statistically different.

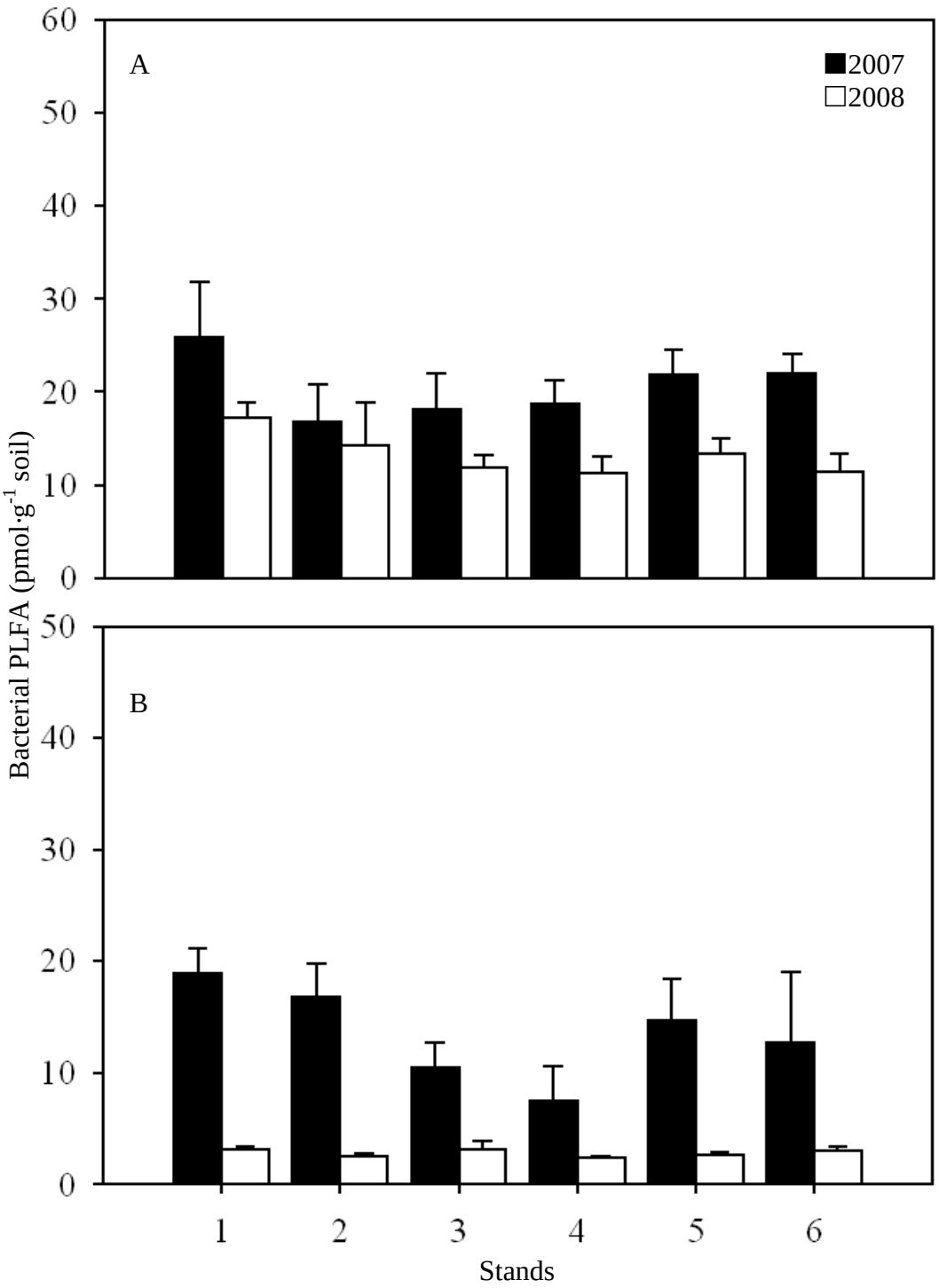

Figure 4.4.11 – The mass of bacterial PLFA biomarkers (pmol·g^{-1}) present in the LFH (A) and mineral (B) layers of all six differently aged lodgepole pine stands of west central Alberta during the June sampling of 2007 and 2008. Each sample consists of approximately nine different sampling points. The error bars presented on this figure are the mean standard error for each stand. See Table 4.3.2 for bacterial PLFA biomarkers.

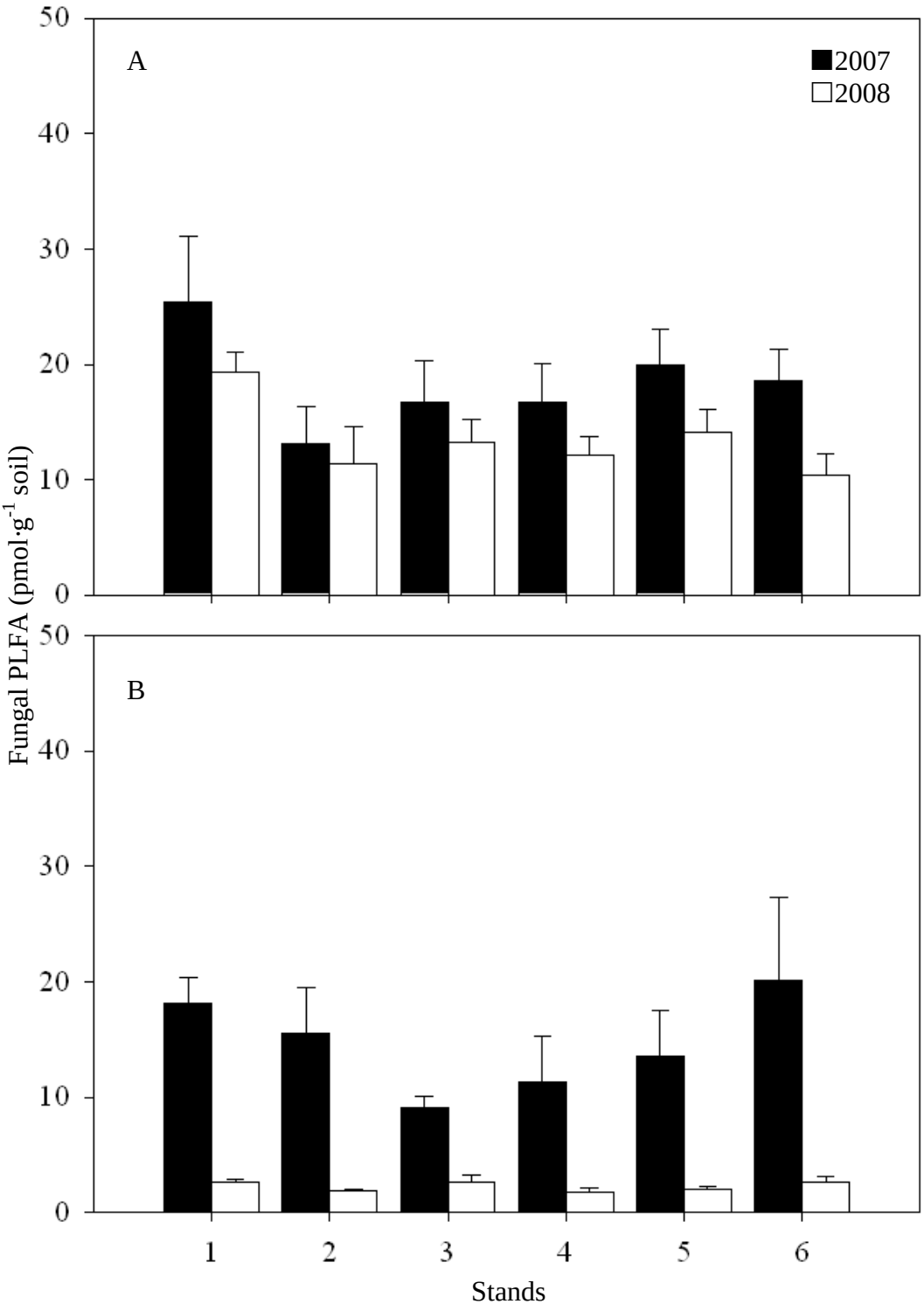

Figure 4.4.12 – The mass of fungal PLFA biomarker (pmol·g^{-1}) present in the LFH (A) and mineral (B) layer of all six differently aged lodgepole pine stands of west central Alberta during the June sampling of 2007 and 2008. Each sample consists of approximately nine different sampling points. The error bars presented on this figure are the mean standard error for each stand. See Table 4.3.1 for fungal PLFA biomarkers.

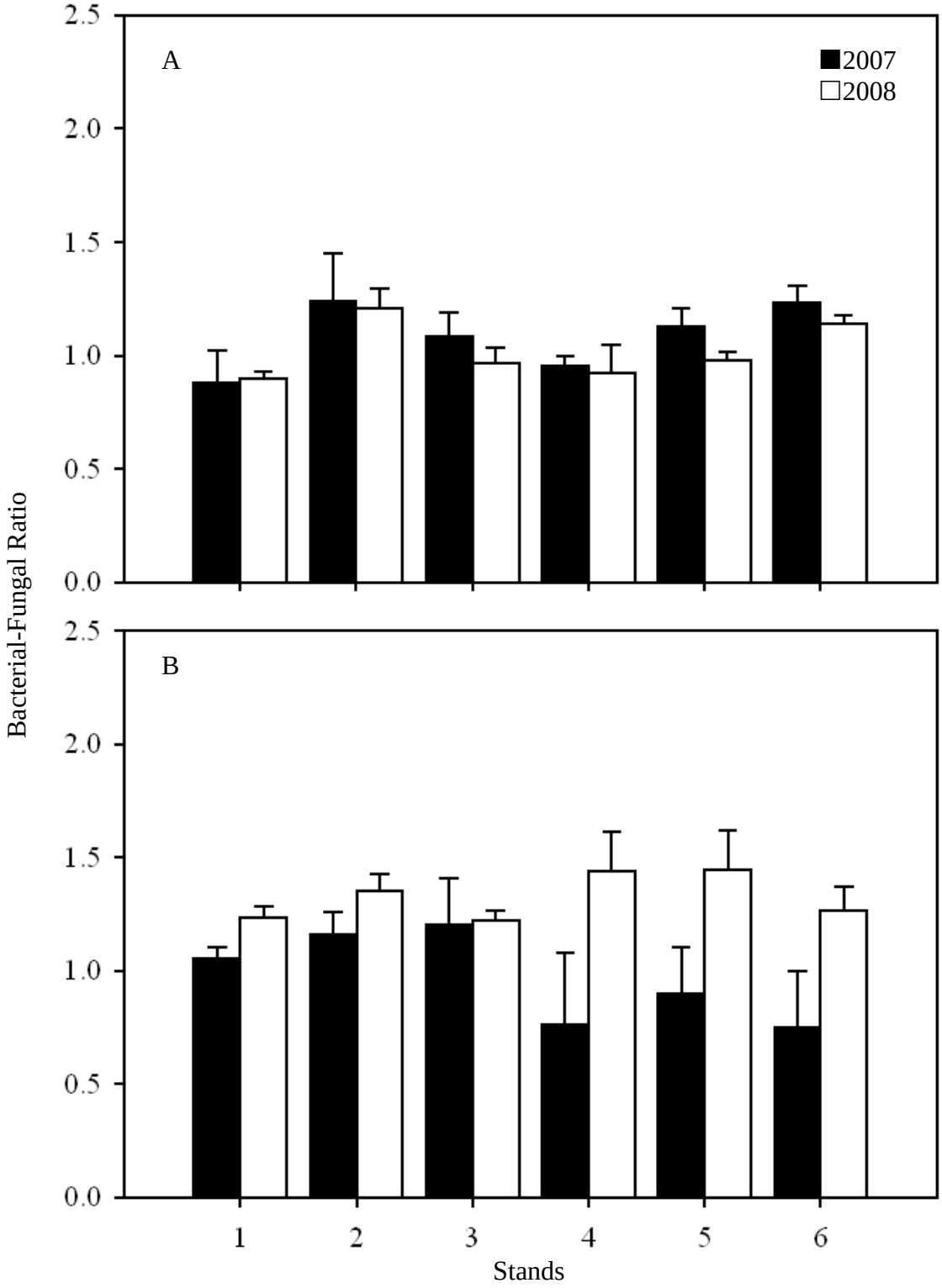

Figure 4.4.13 - The bacterial-fungal ratio in the LFH (A) and mineral (B) layers of all six differently aged lodgepole pine dominant stands in west central Alberta during the June sampling of 2007 and 2008. Each sample consists of approximately nine different sampling points. The error bars presented on this figure are the mean standard error for each stand. See Table 4.3.2 for PLFA biomarkers

4.5 Discussion

Many studies show that microbial communities are influenced by anthropogenic disturbances such as forest harvesting (Nilsson et al., 2007); however, this study showed that harvesting does not have a significant effect on the microbial community. This was determined by examining the microbial communities of different forest stands using CLPP and PLFA as a means of determining the functional and physiological fingerprints of the microbial communities. The microbial communities changed throughout the season in 2007. The June 2008 microbial communities were similar to the microbial communities within the stands during August 2007. The only major difference was found between the soil layers within all the stands.

Structurally, similar microbial communities commonly occur between forest stands. There are many studies that show the phenomenon where microbial community structures do not change after a stand has been harvested (Smolander et al., 1998). This relationship within forest stands, generally, arises in conifer-dominated stands, which make up a large part of Boreal forests (Baath et al., 1995; Fraterrigo et al., 2006; -Pietikainen et al., 2001). These conifer stands contain a thick LFH layer (Table 3.3.1) with a large amount of organic matter that allows for a better buffering capacity within post harvest stands.

This increased buffering capability of the soil prevents drastic shifts in the soil microbial communities. Other studies have shown a shift in surface soil microbial communities after a disturbance event such as harvesting (Gallo et al., 2004). This is the result of a reduced forest floor with a low buffering capacity and less organic matter. As a result, forests dominated by deciduous trees like aspen show a significant difference when one examines pre and post harvest stand microbial communities (Leckie, 2005).

4.5.1 Overall patterns in functional and physical microbial community fingerprints within soil layers

Many studies observe that when the LFH and mineral samples are examined, a clear difference between these two layers is found. (Fierer et al., 2003; Ghiorse and Wilson, 1988; Zvyagintsev, 1994) In most studies, there is a gradual decrease in the microbial community as one goes deeper into the soil until the soil layer changes. The difference in microbial communities at these layers is based on differences in the soil, and the physical and chemical properties within each layer.

The LFH has a large amount of organic matter, while the mineral layer is very low in organic matter and has a texture of sandy clay loam. The chemical and physical differences of these layers cause the microbial diversity and microbial community structure to be very different. The Fierer et al. (2003) reported that microbial activities are different between the soil layers. A large number of microorganisms are involved in decomposition within the surface soils, while in the lower layers, there are limitations and competition (Fritze et al., 2000; Taylor et al., 2002), which result in a reduced microbial population and community.

The limitations within the lower soil layers are associated with their composition. It has been clearly observed that the concentration of organic matter decreases significantly within the mineral soil layers while the density of the soil increases, which leads to a reduction in the amount of pore space (Soil Classification Working Group, 1998). This dramatically affects the amount of oxygen that is able to penetrate the soil. As a result, there will be less oxygen available for the microbes within the layer (Barnes et al., 1998). In addition, if saturation of these layers occurs; this affects the microbial community as it creates an anaerobic condition (Adams et al. 1991). This condition forces the community

to change. Even though this shift occurs, the layer's functional characteristics do not change.

4.5.2 Seasonal variations within the soil microbial community structure of pre and post harvested stands in 2007

There was a significant seasonal variation within the microbial communities of the 2007 sampling period. It was clearly shown that the communities within the LFH of all six stands differed when the microbial communities for the beginning of the sampling season, June 2007, were compared to that of the end of the sampling season, August 2007. The mineral layer's microbial communities within the six research stands did not show significant changes during the sampling season June and August microbial communities were very similar in microbial diversity.

The LFH layer demonstrated a distinct difference in microbial communities between the beginning and the end of the sampling season. On examining what had changed between these two sampling periods, it was found that moisture within the soil had increased over the sampling period. According to the soil moisture data collected, the amount of water was much higher in the August 2007 samples as compared to the amount of water of the June 2007 samples (Table 3.3.2). Previous studies have reported that increased moisture in soil results in a reduction in the microbial communities diversity and populations (Degens, 1998). Keift et al. (1987a) showed this in their study, which examined the change in the microbial community after wetting a dry soil. Wetting the soil enabled them to illustrate that there is a direct relationship between soil moisture and the population of the different microbes in the microbial community.

Therefore, an increase in moisture causes an increase in certain populations within the microbial community independent of the effect of disturbance. As a result, not only was

there a shift within the post harvest stands after increased rainfall, but also a shift in the pre harvest stand. This wetting effect remained consistent within all stands, pre and post harvest because of the ability of conifer stands to remain well buffered from the effect of harvesting (Kieft et al., 1987). This caused the pre and post stands to be similarly affected by the rewetting following a below average precipitation year.

The mineral layer of each site did not show this shift in the functional structure of the microbial communities. These layers remained relatively stable between both sampling periods. There are several reasons for this lack of change in the mineral layer. The general consensus from previous research is that the mineral layer does not receive the additional moisture from the rainfall and the moisture that reaches the mineral layer is drained from these soils relatively quickly. As a result, there is not a drastic shift in the microbial community structure in the soil's mineral layer. On examining the PLFA fingerprint of the microbial community, we noted that a change in its structure did occur; however, this did not alter the functionality of the microbes.

4.5.3 Functional and Physical Microbial Community Composition

The functional communities within the LFH and mineral layers of the soil showed no significant change between stands. This was apparent on examining the total percentage of the six guilds (Table 4.3.1) of interest. There was no difference between the percent of guilds within both years 2007 and 2008 (Figure 4.4.2, Figure 4.4.3). There was, however, an observable difference between the beginning and the end of the 2007 season within the LFH layer. The driving force of this difference was the change in moisture as seen in the previous NMS plot comparisons. The mineral layers remained consistently similar throughout the season, even after seasonal rainfall as noted by their functional structure.

This can be caused by the ability of the LFH layers to absorb a large quantity of water and it can also be due to the soil characteristics of the mineral layers, which allowed a relatively rapid rate of drainage through the soil. However, due to the fact that the actual microbial community shifted, it is more than likely that the groups of organisms changed to a community better adapted to the environment, but that utilizes the same nutrients.

On examining the physiological structure of the microbial community, a drastic shift can be seen in the composition with a rapid reduction in the fungal population of the LFH while there was no observable change in the mineral layers` microbial community. This was similar to that of the microbial communities' comparison in the previous NMS plots, which show these similarities and differences. Therefore, the microbial communities did shift as a result of the increased moisture within the LFH. This increase also caused the fungal population within the community to decrease in all stands.

4.6 Conclusion

To conclude, the microbial communities changed within all six stands during the 2007 sampling periods and remained changed in 2008. These changes are consistent within all stands, which indicate that this is an environmental event rather than the effect of harvesting. The increased rainfall during the 2007 sampling year caused this shift in the microbial functional and physiological community. The changes were determined using CLPP and PLFA respectively. When the pre and post-harvested stands were compared, no change was noted in their microbial community structure. This was due to the high buffering capacity within the conifer stands' LFH soil composition (Kieft, et al. 1987). The mineral microbial community remained unchanged throughout this study

because the moisture remained within the LFH so that there was little or no change of moisture within the mineral soil.

4.7 References

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CHAPTER 5
Summary and Conclusions

Sustainable forest management is a concept, which is changing and developing over time to improve productivity and encourage rapid recovery following harvest. Understanding the global and local biogeochemical cycle allows one to develop a better means of sustaining a forest even if it undergoes several cycles of regrowth and harvest. To improve our understanding of the biogeochemical cycle in relation to forest harvesting, I conducted research on two forest soil characteristics: nutrients, in the form of nitrogen and phosphorus, and microbial communities. My study attempted to understand the change in nutrients and the microbial community structure in pre and post harvested lodgepole pine stands in west central Alberta. As soil conditions change throughout the seasons, the relationship between soil nutrients, microbial communities and stand age becomes more and more complex and hence difficult to examine.

5.1 Nutrient availability within the study area within pre and post harvest stands

This was a multiple stand study that examined five different forest stands for nutrient availability and six stands for microbial community structure. Nutrient measurements were taken using PRS probes, and the availability of two macronutrients was examined: phosphorus (P as PO_4^{3-}) and nitrogen (N as NO_3^- and NH_4^+). In previous forest-harvesting studies, it was observed that nitrogen in the form of ammonium is readily released from the system, which is caused by the breakdown of organic compounds (Tate

and Salcedo, 1988; Piirainen et al., 2002). My study, however, shows there were no increases in the NO_3^- flux to the PRS probes and only a small increase in NH_4^+ flux in the mineral layer of forest soils. This occurred because the lodgepole pine stand had a large LFH that absorbed the NO_3^- and NH_4^+ and immobilized these nutrients. In the mineral layer there was a slight increase in ammonium PRS flux as little to no absorption occurs within this layer.

The phosphorus PRS flux data revealed a change in phosphorus levels within the LFH that decreased as stand age increased. There was minimal phosphorus PRS flux within the mature stand, but once harvested this flux increased significantly. Furthermore, after this significant flux there was a gradual decrease of phosphorus as the stands aged, resulting in phosphorus PRS flux similar to that of the mature stand. This indicates that changes, like the decomposition of organic matter within the LFH post harvest caused an increase in phosphorus availability. The mineral layer, however, remained fairly constant throughout all the pre and post harvested stands. I propose the increase in phosphorus within the LFH was probably caused by the degradation of dead plant tissues that releases large quantities of phosphatase as dead cells rupture.

5.2 Microbial community structure within the six differently aged Lodgepole Pine stands

Forest harvesting is a major disturbance to the forest soil environment. Many studies have shown that the microbial communities change once a harvesting event has occurred (Chatterjee et al., 2008). These communities show some change in their functional and physiological characteristics. It was clearly shown in my study that, within the LFH, the functional characteristics of microbial communities as measured by CLPP changed in

response to seasonal changes, However, the microbial community physiological characteristics, measured by PLFA, remained the same. This was the complete opposite for the mineral layer, where no shift occurred in the functional characteristics of the microbial community, but a very distinct shift occurred in the community's physiological characteristics. The changes in physiological and functional characteristics were not due to harvesting, but were due to seasonal change and environmental factors over a single growing/sampling period. Furthermore, the change may have been due to the increased rainfall over the two years of sampling. For example, the two years prior to the initiation of this study there was below average rainfall, whereas in 2007 and 2008 sampling years there was average and above average moisture (Section 3.3.1.1)

5.3 Overall relationship of nutrients and the microbial community

In conclusion, this study determined there was no clear direct relationship between phosphorus (P), ammonium (NH_4^+) and nitrate (NO_3^-) and changes in the microbial communities within the stands. The phosphorus flux to PRS probes was related to harvesting of a stand, whereas no observable differences between the microbial communities in these stands were detected. Seasonal changes were observed for the microbial community functional characteristics but there was no corresponding change in the phosphorus PRS flux. Previous studies show similar results where there is an increase in phosphorus within a forest floor and soil respiration increases but the microbial biomass did not change (Tate and Salcedo 1988; Gobran et al. 1998; Allen and Schlesinger, 2004). This may be due to the fact that phosphorus is being bound to Al, Fe or even adsorbed to the clay within the soil (Zou et al. 1997).

Ammonium flux to PRS probes within the mineral layer increased when harvesting occurred but there was only a seasonal change in the microbial community. Thus there was no relationship between changes in the microbial community structure and NH_4^+ PRS flux in this study. In contrast, the NO_3^- PRS flux did not change during the season or due to harvesting whereas there was a shift in the microbial communities functional and physiological characteristics during the season. Thus, providing a clear indication that there was no relationship between NO_3^- and microbial community. Previous studies show that nitrogen in the forms, NO_3^-, and NH_4^+, do influence the physiological and functional characteristics of the microbial community (Allen and Schlesinger, 2004). However NO_3^- and NH_4^+ may become available within the stands after harvesting but are being rapidly taken up and immobilized by the ground vegetation (Carmosini et al. 2003), which reduces the available nutrients in the system.

My results indicate microbial community changes occur within forest stands after harvest. Furthermore, P and N availability also change as organic matter is decomposed. There was, however, no clear relationship between the general microbial community and N and P flux to PRS probes. Thus additional studies are needed to determine if specific microbial communities such as nitrifying bacteria changed in these forest stands after harvesting. One important finding of my study was that current-harvesting practices did not alter the microbial community significantly whereas environmental factors did. Further research on nutrient transformations and microbial community characteristics within lodgepole pine forest stands are needed to provide a clearer picture of their relationships.

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APPENDICES

APPENDIX A

SITE DESCRIPTIONS AND SAMPLING DESIGN FOR NUTRIENTS AND SOIL SAMPLING.

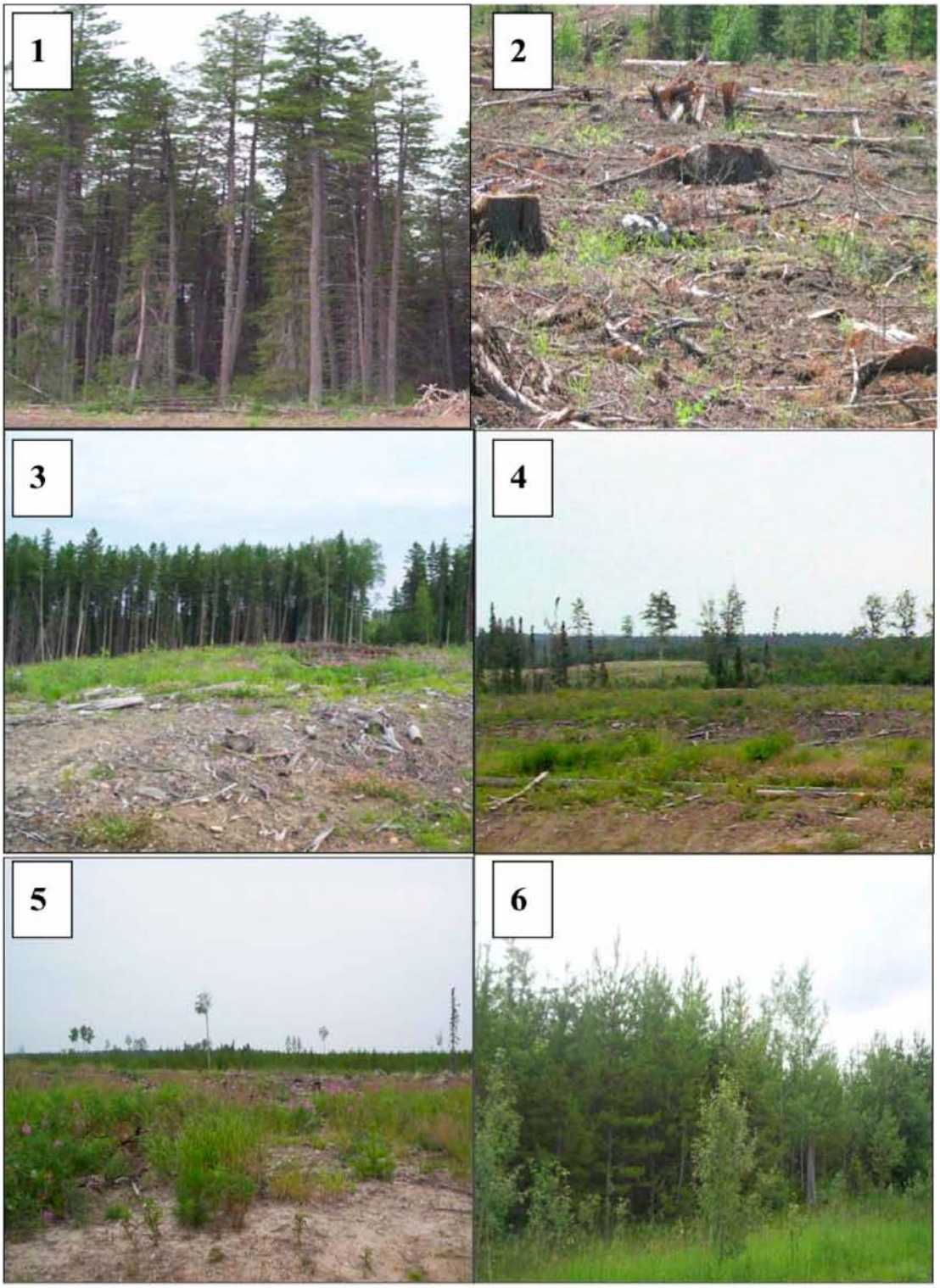

Figure A.1 – The six different lodge-pole pine harvested stands. The image numbers correspond to the stand designation and descriptions in table 3.3.1.

Figure A2 – The Burial of PRS probes within the mineral layer of the soil profile of Stand 4 during the June sampling period. The LFH layer of soil was removed to expose the mineral layer. Two probes, and orange/red and purple probe, were inserted into the soil. The probes are positively (cation) and negatively (anion) charged, respectively.

Table A.1– Average flux measurements of cations and anions from the PRS probes analysis. (n=9) The data presented in this table is in $\mu g \cdot 10 cm^{-2} \cdot (12 \text{ weeks})^{-1}$.

	2007					2008				
Stands	1	2	3	4	5	1	2	3	4	5
LFH										
Ca	1718.0	3427.4	3760.0	1933.2	2128.4	1165.3	3582.6	2304.5	2217.7	2292.7
Mg	356.6	507.7	582.7	420.6	457.4	250.3	509.9	460.6	436.8	477.1
K	1054.9	316.3	325.4	495.8	527.0	759.7	460.3	645.0	430.3	452.2
Fe	10.9	76.5	79.9	19.8	47.3	4.8	25.5	30.3	35.9	45.2
Mn	202.3	101.4	109.4	186.3	226.5	144.3	89.8	146.6	208.8	218.4
Cu	0.2	0.2	0.2	0.2	0.1	0.1	0.0	0.1	0.1	0.1
Zn	8.6	9.9	10.7	9.1	10.7	4.4	6.6	8.5	8.6	8.8
B	2.2	2.9	3.6	2.3	2.7	2.3	2.3	2.5	1.8	2.2
S	88.5	115.0	58.8	59.5	66.0	33.1	60.2	42.0	61.5	69.0
Pb	0.2	0.2	0.2	0.2	0.2	0.0	0.0	0.1	0.2	0.1
Al	85.7	86.2	97.8	89.5	102.3	38.1	61.6	66.0	81.1	93.8
Cd	0.3	0.2	0.2	0.2	0.4	0.0	0.2	0.1	0.2	0.3
Mineral										
Ca	2029.8	3416.7	3444.0	2640.8	2512.7	2333.4	4509.5	2550.8	3065.0	2897.3
Mg	357.8	485.1	497.9	493.8	469.0	381.0	458.2	454.1	505.8	431.1
K	575.7	309.3	215.1	374.3	390.7	310.3	87.2	310.2	311.2	283.9
Fe	109.8	201.2	379.2	136.1	180.2	40.9	299.6	1087.5	528.9	519.3
Mn	141.8	95.4	94.3	197.1	179.8	72.1	71.2	101.1	231.6	133.0
Cu	0.2	0.5	0.2	0.1	0.0	0.2	0.3	0.2	0.2	0.0
Zn	8.9	7.8	8.2	8.5	10.3	6.7	5.6	6.7	9.8	9.8
B	2.5	3.0	2.9	2.5	2.5	2.1	2.6	2.0	2.4	2.3
S	198.2	126.2	60.1	110.4	86.7	97.0	105.7	81.7	113.0	112.2
Pb	0.3	0.5	0.6	0.3	0.3	0.1	0.5	0.5	0.4	0.3
Al	137.4	110.3	128.2	128.7	126.9	124.6	100.8	152.0	170.3	144.5
Cd	0.2	0.2	0.1	0.2	0.3	0.2	0.2	0.2	0.3	0.2

Figure A.3 – A soil sampled and the areas of separation between the LFH and mineral layers. Samples were separated by organic and non-organic samples. This caused the samples to be designated as layers rather then horizons.

APPENDIX B

UTILIZATION OF NON-PARAMETRIC MULTIDIMENTIONAL ANALYSIS TO DETERMINE MICROBIAL COMMUNITY STRUCTURE

The use of a non-parametric multi-dimensional scaling (NMS) analysis was to allow for a more robust analysis of the data set without making major assumptions, like normality or homogeneity of the data. This coupled with MRPP, pairwise comparison and analysis of variance (ANOVA) provides a detailed description of the samples relationships between sites, stands and periods of sampling. The MRPP provides information about the relative relationship between stands and periods, while the pairwise comparison provides additional information about the stands relationship. The ANOVA is a method to determine the variance found within each axis of the two most dominant dimensions within the NMS plot.

Table B.1 – The final configuration for the CLPP analysis, axis 1 and 2, for the June 2007 and 2008 LFH samples obtained form the PC-Ord program using NMS analysis.

	2007		2008	
Stand	Axis 1	Axis 2	Axis 1	Axis 2
6	0.73728	-0.01704	-1.15892	0.08162
6	0.74659	-0.11076	-0.15542	0.98706
6	0.84507	0.11881	-0.1625	0.95645
6	-0.01962	-1.77913	-0.24123	0.84847
6	1.20244	0.61213	-0.50335	0.31607
6	0.5585	-0.47949	-0.71001	0.20298
6	0.32021	-0.92983	-1.0891	0.0576
6	0.48679	-0.56294	-1.1927	-0.27161
1	0.87553	-0.01727	-1.3724	-0.77876
1	0.85182	0.0716	-0.64818	0.30097
1	1.17692	0.3326	-0.53831	0.46878
1	1.24941	0.44565	-0.43671	0.5588
1	0.77621	-0.25701	-0.53751	0.42091
1	0.8954	0.02268	-0.63925	0.45635
1	0.38599	-0.66604	-0.48077	0.67722
1	0.80541	0.04518	-0.88337	-0.00057
1	0.96692	0.18868	-0.80324	0.084
2	0.76259	-0.13283	-1.27605	-0.36537
2	0.37294	-0.96967	-0.74104	0.17044
2	0.88438	-0.14166	-0.17947	1.00944
2	0.59914	-0.46969	-0.69447	0.15801
2	-0.85377	-3.2428	-0.79405	0.33477
2	0.6117	-0.27323	-0.57762	0.35695
2	0.60277	-0.1959	-1.12086	-0.14332
2	0.96116	0.01413	-0.71865	0.10433
2	0.48992	-0.60012	-0.85793	0.11918
3	0.77029	-0.23308	-0.81	0.04581
3	0.70097	-0.27379	-0.41129	0.70908
3	0.59172	-0.40684	-0.50693	0.38919
3	-0.02661	-1.28815	-0.40261	0.66307
3	0.52506	-0.50429	-0.50157	0.44899
3	0.86035	-0.08569	-0.98011	0.21862
3	0.83531	-0.21835	-0.24403	0.62635
3	0.86899	0.03878	-0.38096	0.5388
3	-0.06046	-1.50022	-0.51481	0.7178
3	0.76315	-0.19837		
4	0.86074	-0.06549	-0.34647	0.78287
4	1.08785	0.17364	-0.4716	0.59819
4	0.73903	-0.27634	-1.5202	0.04098
4	0.82142	-0.06797	-0.20873	0.76723
4	-0.41279	-2.22656	-0.54749	0.22469
4	0.80876	-0.13396	-0.48303	0.45535

Table B.1 continued.

4	0.87919	-0.04026	-0.8108	-0.19012
4	0.74782	-0.12133	-1.40843	-0.34949
4	0.87788	-0.00023		
5	0.76872	-0.1621	-0.41059	0.66391
5	1.17255	0.28748	-0.65149	0.25796
5	-1.08908	1.42562	-0.3541	0.65964
5	0.83417	-0.03135	-0.32693	0.84017
5	0.72923	-0.18681	-0.79151	-0.01051
5	0.63432	-0.41851	-0.49655	0.47458
5	1.08428	0.13819	-0.59151	0.37889
5	0.4808	-0.77861	-0.77444	0.05644
5	0.48737	-0.51267	-1.17347	-0.45801

Table B.2 - The final configuration for the PLFA analysis, axis 1 and 2, for the June 2007 and 2008 LFH samples obtained form the PC-Ord program using NMS analysis.

	2007		2008	
Stand	Axis 1	Axis 2	Axis 1	Axis 2
1	0.9317	-1.9612	-0.2756	-0.2262
1	0.8515	-1.7428	-0.1138	0.296
1	0.7604	-1.1283	-0.0933	0.2272
1	0.3188	-0.4414	1.1386	-2.9986
1	0.6196	-1.0836	-0.1497	-0.006
1	0.9467	-1.2923	-0.5455	0.3721
1	-0.0029	0.7558	0.0835	-0.5039
1	-0.5261	0.5652	-0.2842	0.3859
2	-0.0344	0.5233	-0.2672	0.3322
2	0.1882	0.6793	-0.3761	0.5919
2	0.1407	0.0585	0.2231	-1.2341
2	0.0175	0.2682	-0.4111	0.2633
2	1.0428	-2.442	-0.3145	0.5144
2	0.3003	-0.9797	-0.1217	-0.0913
2	N/A	N/A	-0.1917	0.0287
2	N/A	N/A	-0.275	0.1952
2	N/A	N/A	-0.4169	0.2299
3	-0.3053	0.788	-0.1534	-0.1419
3	-0.0807	0.6137	-0.1506	-0.374
3	-0.2381	1.0649	-0.579	0.7526
3	0.3293	0.2698	-0.313	-0.0114
3	-0.1228	0.5367	-0.3124	0.2738
3	-0.1738	0.6024	-0.4513	0.43
3	0.3947	-0.633	2.0181	0.8556
3	0.984	-2.079	-0.6376	0.9307
4	0.4602	-0.2016	-0.3341	0.3188
4	0.7897	-1.3757	-0.3876	0.5585
4	0.6841	-1.2119	-0.2867	0.3752
4	0.0329	0.5536	-0.3741	0.4989
4	-0.2744	0.7853	-0.2459	0.4229
4	-0.0156	0.3389	-0.5031	0.3601
4	-0.2369	0.8613	0.5859	-1.9716
4	-0.0537	0.4509	-0.3503	0.1909
4	-0.385	0.4722	-0.535	0.8052
5	0.0454	0.4358	0.0599	-0.032
5	0.246	0.3028	0.4338	-1.3815
5	0.7175	-1.241	-0.2769	0.6666
5	0.7034	-1.3951	-0.2743	0.3983
5	-0.6184	1.2337	-0.1807	0.4898
5	0.1326	-0.4687	N/A	N/A
5	-0.1268	-0.0375	N/A	N/A
6	-0.1623	0.5624	-0.5818	0.6988
6	-0.069	0.5169	-0.2583	-0.3655

Table B.2 continued				
6	-0.1608	0.6055	-0.4524	0.4739
6	-0.0035	0.3791	-0.4965	0.203
6	-0.2406	0.6716	-0.3887	0.592
6	-0.0847	0.6059	-0.7907	1.045
6	0.8558	-1.2671	-0.7722	0.8723
6	0.6561	-0.841	-0.4491	0.5712
6	0.9879	-1.0812	-0.3925	0.5196

Table B.3 – Multi response permutation procedure (MRPP) results for the determining the relationships between the six different stands and two different years for PLFA analysis of the LFH soil layer. “A” represents the variation within the groups and the P-value indicates if the stands are different then one another.

Stands	Average Distance	A	P-value
1	0.40811822		
2	0.32876917		
3	0.49850355		
4	0.62680509		
5	0.47768424		
6	0.29197531		
Comparison			
1vs4		0.2594217	0.00053782
1vs3		0.15432099	0.00093506
1vs2		0.06595191	0.03655435
1vs6		0.11176088	0.01533707
1vs5		0.18177388	0.00174045
2vs6		0.01153346	0.25612488
2vs5		0.07001299	0.00809932
3vs2		-0.00519818	0.53090475
3vs6		0.02306693	0.11476136
3vs5		0.06595191	0.00920543
4vs3		-0.00893437	0.61422522
4vs2		0.04207277	0.06139718
4vs6		0.04580897	0.03937236
4vs5		0.07716049	0.00676971
6vs5		0.01104613	0.26877132
Years			
2007	0.57183342		
2008	0.303098		
2007vs2008		0.11739042	0